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CONTENTS

Page.

The Metabolism of Ascorbic Acid in the Horse, P. B. Pearson, M. K. Sheybani and H. Schmidt 175

Clean Wool Yield Variation Among Regions of Rambouillet Fleeces, Elroy M. Pohle, H. W. Wolf and Clair E. Terrill 181

Fiber Density and Some Methods of Its Measurement in the Fleeces of Rambouillet Sheep, H. W. Wolf, W. M. Dawson and E. M. Pohle 188

Wool Fineness in Eight Sampling Regions on Yearling Rambouillet Ewes, Elroy M. Pohle and R. G. Schott 197

The Value of Alfalfa and Certain of Its Fractions in the Nutrition of Breeding Ewes, C. L. Shrewsbury, F. N. Andrews, Claude Harper and M. R. Zelle 209

Variations in the Occurrence of Bloat in the Steer Progeny of Beef Bulls, Bradford Knapp, Jr., A. L. Baker and Ralph W. Phillips 221

Determinations of Metabolizable Energy of Feeding Stuffs for Cattle, E. B. Forbes and E. J. Thacker 226

A Study of the Characteristics of Live Market Hogs as Related to the Quality of Carcasses Produced, J. P. Willman and J. L. Krider 231

Chemical Composition, Grazing Value and Vegetative Changes of Herbage in a Typical Plains Pasture, Warren N. McMillen, Quentin Williams and Wright Langham .. 237

The Preparation of Muscular Tissues for Histological Study, Eunice Johnson, F. N. Andrews and C. L. Shrewsbury 244

Induction of Estrus and Ovulation in Ewes During Their Anestrus Season, A. H. Frank and Aaron Appleby 251

News and Notes 259

THE METABOLISM OF ASCORBIC ACID IN THE HORSE[1]

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WHILE it is generally agreed that ascorbic acid is not a dietary essential for the horse, information on its metabolism in this species is meagre. Following the recent observations by Phillips and Lardy (1940) and Phillips *et al.* (1941) on the relationship of ascorbic acid to reproduction in the bovine, the desirability of ascorbic acid studies with the horse became increasingly apparent. The present paper reports the ascorbic acid values of blood plasma and the amount excreted daily in the urine of horses maintained on a controlled diet, and following the administration of large doses of ascorbic acid.

Experimental

Shetland ponies were used because of the ease of handling them and collecting the urine in the metabolism cage. These animals were being used concurrently in investigations on certain members of the vitamin B group. The percentage composition of the basal rations was dried beet pulp 60, yellow corn 29, casein 10, salts 1, and vitamin A and D concentrate. This ration was altered during the course of the experiment to include brewers' yeast or certain of the B vitamins in synthetic form. The fact that neither the addition nor exclusion of the B vitamins from the ration had no effect on the ascorbic acid content of the blood or urine constitutes confirmatory evidence that the changes in the dietary regimen had no pertinent influence on the phases of ascorbic acid metabolism studied. The animals were maintained on the experimental rations for about three months before starting the ascorbic acid studies reported in this paper.

Blood and urine samples were collected at approximately monthly intervals. The blood was drawn from the jugular vein and run into conventional oxalated tubes. The urine was collected quantitatively by using a specially constructed metabolism cage with urinary funnel and tube. The glass container in which the urine was collected was painted black to exclude the light and enough glacial acetic acid added to make the urine distinctly acid. The ascorbic acid determinations were made by the method of Mindlin and Butler (1938).

[1] Published with the approval of the Director of the Agricultural Experiment Station as technical contribution N. 753. The authors are greatly indebted to Merck & Co. for a generous supply of ascorbic acid used in these experiments.

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Ascorbic Acid in Plasma and Urine

The ascorbic acid content of the plasma and the average daily urinary excretion of the various animals is recorded in table 1. The average plasma ascorbic acid was 0.32 mg. per cent. This is considerably less than 0.73 mg. per cent reported by Errington *et al.* (1942), but is significantly higher than 0.12 mg. per cent reported by Davis and Cole (1942). It is quite possible that these differences are due at least in part to the analytical methods used. It is well recognized that a sharp end point in the indophenol titration method is difficult to secure. The photoelectric method eliminates this difficulty. The data reported in this paper undoubtedly represent a much more homogeneous group of animals than the data in either of the other papers.

TABLE 1.—ASCORBIC ACID CONTENT OF EQUINE PLASMA AND URINE

Animal No.	Number of observations	Ascorbic Acid		
		Plasma	Urine	
		mg. per cent	mg./24 hrs.	mg./kg. wt./24 hrs.
1	6	0.39	35.2	0.27
2	6	0.29	41.3	0.36
3	6	0.33	48.8	0.36
4	6	0.26	27.9	0.19
5	6	0.35	69.2	0.67
Average*	30	0.32±0.13	44.4±25.8	0.37±0.25

* Average ± standard deviation.

Nevertheless, the wide variation between 0.73 and 0.12 mg. per cent strongly suggests that factors other than the true ascorbic acid content of the plasma are responsible for this difference. The individual plasma ascorbic acid values for the various animals showed some variation as evidenced by the standard deviation of 0.13 mg. per cent. This represents a much smaller variation than the range of 0.30 to 1.76 mg. per cent reported by Errington *et al.* (1942).

The average daily urinary excretion of ascorbic acid for the 30 observations on the five ponies was 44.4 mg. or 0.37 mg. per kg. of body weight. The averages for the individual animals ranged from 27.9 to 69.2 mg. daily. There was considerable variation in the individual values for the various animals and this is reflected in the relatively large standard deviation of 25.8 mg. These animals were receiving a ration that furnished not more than a small amount of ascorbic acid. The horses used in these investigations ranged in weight from about 102 to 145 kg. It is of interest to compare the ascorbic acid excretion of these animals with that of man. The daily ascorbic acid

excretion of man on a diet adequate in this respect has been reported as 15 to 30 mg. The daily urinary excretion of ascorbic acid by cows on a standard ration ranged from 26.8 to 54.1 mg. (Knight *et al.*, 1941) when determined by the indophenol titration method. On the basis of body weight this is considerably lower than the amount excreted by the horse.

Effect of Feeding Ascorbic Acid

In studying the effect of the ingestion of ascorbic acid on the amount in the plasma and the amount excreted, the animals were kept in the metabolism cage for 48 hours. The first 24-hour sample of urine was analyzed for ascorbic acid and this is recorded as the initial value in table 2. Blood for plasma ascorbic acid determinations was drawn at the end of the same period. The

TABLE 2.—EFFECT OF THE INGESTION OF SIX GRAMS OF ASCORBIC ACID ON AMOUNT IN PLASMA AND URINE

Animal No.	Number of observations	Plasma ascorbic acid		Urinary ascorbic acid	
		Initial	After ascorbic acid	Initial	After ascorbic acid
		mg. per cent	mg. per cent	mg./24 hrs.	mg./24 hrs.
1	3	0.31	0.37	39.2	104.4
2	3	0.20	0.35	35.7	81.5
3	3	0.42	0.66	41.1	165.0
4	3	0.23	0.33	20.9	27.8
5	3	0.28	0.50	57.2	179.3
Average*	15	0.29±0.14	0.44±0.21	38.8±19.7	111.6±73.0
Increment			0.15		72.8

* Average ± standard deviation.

animal was then fed six grams of synthetic ascorbic acid mixed with the feed. The urine was collected during the next 24 hours and a sample of blood drawn at the end of the period. The data collected in this series were treated statistically, but for the sake of brevity only the important figures are included in the paper. There was no significant difference in the manner in which each of the animals responded to the feeding of ascorbic acid, either in respect to the amount excreted in the urine or the plasma values.

Twenty-four hours after feeding six grams of ascorbic acid there was an average increment of 0.15 mg. per cent in the level in the plasma. The difference between the average initial value and the value after feeding the ascorbic acid is highly significant as judged by a t value of 3.97. This strongly suggests that a part of the ingested ascorbic acid was absorbed and that it was not completely eliminated during the period of observation. This is in contradistinction to the conclusions of Errington *et al.* (1942). On the basis

of blood plasma studies following the ingestion of ascorbic acid they concluded that little or none of the vitamin is assimilated by the horse when fed.

Considering now the ascorbic acid content of the urine we see that there was an average increment of 72.8 mg. in the daily excretion following the ingestion of six grams of the vitamin. This increase is highly significant according to Fisher's *t* test. These data would seem to leave little doubt but what a part of ingested ascorbic acid is assimilated by the horse. The per centage recovery in the urine of the amount ingested is, however, very small. This indicates that there is substantial destruction of ingested synthetic ascorbic acid in the alimentary tract of the horse, probably in a manner similar to that which occurs in the cow. Knight *et al.* (1941) failed to find any increase in the ascorbic acid of the plasma, milk or urine after feeding massive doses of the vitamin to cows. Since the bacterial action is much more extensive in the stomach of the cow than in the stomach of the horse, this difference in the destruction of ingested synthetic ascorbic acid by the two species is not surprising.

Injection of Ascorbic Acid

The excretion of ascorbic acid administered intravenously was studied for the purpose of comparing it with the urinary recovery following oral administration. Six grams of ascorbic acid was dissolved in about 20 ml. of sterile water and injected into the jugular vein. The urine was collected for a period of 24 hours prior to administering the ascorbic acid. Blood samples were drawn just prior to giving the ascorbic acid and again 24 hours later.

The excretion of injected ascorbic acid over a 24-hour period and the amount in the plasma at the end of the period are shown in table 3. Only one

TABLE 3.—THE EFFECT OF INTRAVENOUS INJECTION OF SIX GRAMS OF ASCORBIC ACID ON AMOUNT IN PLASMA AND URINE

Animal No.	Plasma ascorbic acid		Urinary ascorbic acid			
	Initial	After ascorbic acid	Initial	After ascorbic acid	Recovered	
	mg. per cent	mg. per cent	mg./24 hrs.	mg./24 hrs.	mg.	per cent
1	0.50	0.26	8.6	2390.2	2381.2	39.7
2	0.36	0.78	38.8	3022.2	2983.4	49.7
3	0.21	0.29	64.4	1925.9	1861.5	31.0
4	0.16	0.23	20.1	3058.0	3037.9	50.6
5	0.27	0.54	27.0	4664.0	4637.0	77.3

injection was made with each animal, and, as might be expected, there was considerable variation in the amount excreted in the urine. The per cent of injected ascorbic acid recovered in the urine ranged from 31.0 to 77.3. Knight *et al.* (1941) report that a very large per cent of ascorbic acid injected intravenously into cows was recovered in the urine. However, they did not give figures for individual animals so that the data on cows cannot be compared with that for horses. Following the intravenous injection of one gram of ascorbic acid in humans, 50 per cent was recovered in the urine within 24 hours (Wright *et al.*, 1937).

Four of the five animals showed an increased ascorbic acid content of the plasma 24 hours after injection of the vitamin. This indicates that the injected ascorbic acid was not completely eliminated from the body during the period of observation. Had the collection of urine been continued over a longer period it is probable that a larger amount of ascorbic acid would have been recovered. However, it is not likely that the increased amount excreted would have been large as it has been shown that the peak of excretion of injected ascorbic acid in man occurs within one to three hours (Ralli *et al*, 1937; Wright *et al.*, 1937).

The observed increase in plasma ascorbic acid is in accord with the observation of Errington *et al.* (1942). They administered large amounts of ascorbic acid intravenously to two horses. Twenty-four hours later the plasma ascorbic acid values had increased from 0.73 to 1.03 mg. per cent and from 0.68 to 0.84 per cent in the respective animals. In the present study there was no consistent relationship between the increase in plasma ascorbic acid and the amount excreted in the urine during the 24 hours following injection. A larger number of observations would be necessary to determine if any such relationship exists.

Summary

Studies were made on the ascorbic acid values of equine plasma, the amount excreted daily in the urine, and the effect of the ingestion of large amounts of the synthetic vitamin. The ascorbic acid content of the plasma of Shetland ponies on a controlled diet was 0.32 mg. per cent and the daily urinary excretion was 0.37 mg. per kg. of body weight. Following the ingestion of six grams of ascorbic acid there was a significant increase in the amount excreted in the urine during the next 24 hours. At the end of this period the ascorbic acid level of the plasma was significantly higher than the original value. While only a small percentage of ingested ascorbic acid is excreted in the urine, there is evidence that the synthetic vitamin given per os is assimilated to a limited extent by the horse.

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CLEAN WOOL YIELD VARIATION AMONG REGIONS OF RAMBOUILLET FLEECES

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CLEAN wool yield is one of the most important economic considerations to the sheep breeder, wool buyer and manufacturer in appraising the value of the individual fleeces or an entire clip of wool. In breeding for sheep improvement it is often desirable to obtain the yield of clean wool on individual sheep. The scouring of whole fleeces to obtain the yield of clean wool is a time-consuming task, with limited equipment, and therefore, where large numbers of animals are involved, it is usually more practical to depend on scouring results from a small sample of each fleece. It is important to obtain a small sample which is as representative as possible of the entire fleece.

Various methods of sampling fleeces for clean yield determinations have been undertaken by wool investigators but very few have dealt with variation in samples from specific body regions compared with the entire fleece.

Jones and Lush (1927), Wilson (1929), Burns (1931), Buck and LeCompte (1941), Esplin, Phillips and Madsen (1941), Wollner (1941) and Wollner and Tanner (1941) reported on clean wool yield by using small samples and different sampling methods. These determinations were made primarily for determining clean yield in commercial clips or bales of wool. Burns (1940) reported results on the comparison of clean woold yield for different body fleece sorts and found the side and back sorts most representative of the entire fleece. He further found that a combination of the shoulder, side and back fleece sorts gave results very close to that of the entire fleece. Spencer, Hardy and Brandon (1928) made comparisons between wool from different body locations in the Rambouillet, Corriedale and Columbia breeds and found that the side wool averaged approximately the same per cent of clean wool as the whole fleece. Davis, Jones and Warwick (1938) found a high correlation between clean yield in samples of fine wool from the shoulder compared with that for the entire fleece. Wilson (1938) presented a method of satisfactorily obtaining and scouring small samples from fleeces after being run through a fleece opener. Esplin *et al.* (1941) determined clean yield from side samples of wool and found a wide variation between individual animals.

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Schott, Pohle, Spencer and Brier (1942) found a high relationship of side samples with the entire fleece in four breeds of sheep. Pohle (1942) has shown close relationships between side samples both at weanling and yearling ages. Ensminger[2] found a highly significant difference in percentage clean wool yield between individual samples from 9 different body areas in the Shropshire and Southdown breeds. Grandstaff (1942) compared clean yield results in samples from 6 different regions obtained from yearling Navajo and crossbred sheep and found the highest relationship between the shoulder and side with the mean of the other areas.

Previous work has been concerned largely with sampling to obtain the clean yield of clips of wool. Some investigators have compared the clean wool yield of body sorts and more recent work has dealt with variations among samples taken from various body regions. More work is needed on the relative reliability of possible sampling regions on the live sheep in order to perfect a sampling method which will give the most representative clean yield of the entire fleece.

The purpose of this study is to determine the variation in yield of clean wool among regions within the fleece, and the relative reliability of the various regions for predicting the clean wool yield of the entire fleece.

Material and Methods

Small samples were taken from 8 different body regions on 30 Rambouillet yearling ewes during May, 1939, at the Western Sheep Breeding Laboratory, Dubois, Idaho. These 30 ewes were selected from 104 Rambouillet yearling, ewes, born during May, 1938. They had been raised under range conditions typical of the Intermountain West and were grazed on the spring, summer, fall and winter ranges the entire year excepting for about three months during the worst of the winter at which time they were in the feed-lot. Selection of the ewes to be sampled was made at random within groups having the same average fineness at the side as determined by estimated spinning counts. Spinning counts were estimated by three judges working independently and the average of the three was used for each ewe.

The small samples were taken from the following 8 regions on each sheep as illustrated in figure 1: withers, (at top of withers); back, (middle of back); rump, (on back, just anterior to the dock); shoulder, (over mid-point of scapula); side, (at middle of side); hip, (over point of hip bone); on thigh, (even with underline); and on the belly, (directly below the side sample and even with the underline where the belly wool begins but not directly at the mid-line of the belly). The wool was clipped, with electric clippers as close to the skin as possible, from an area approximately 11 centimeters long and

[2] Ensminger, Marion E. Unpublished Theses data—1941. Univ. of Minn. Library.

5 centimeters wide and was immediately placed in a moisture proof container. Each sample contained from 25 to 35 grams of unscoured wool.

The 240 samples were scoured in the Bureau of Animal Industry's animal fiber laboratory at Beltsville, Maryland, by the method described by Hardy (1934). The entire fleeces were scoured by the Agricultural Marketing Administration, Washington, D. C., by the method described by Buck (1940). The wool samples were dried in a conditioning oven at 212° F. for

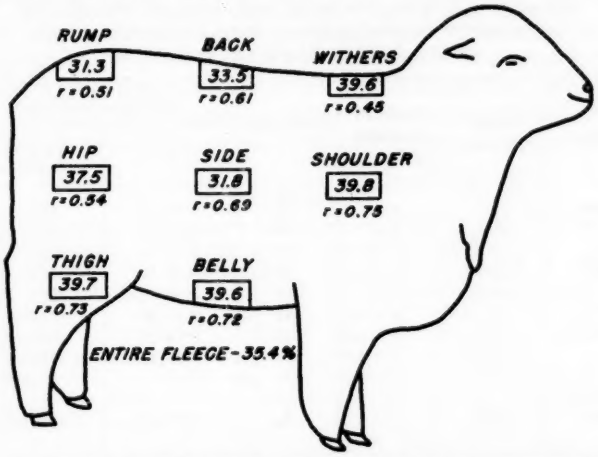

Figure 1. Regions samples, clean wool yields in percentage of bone-dry wool and correlation coefficients between clean wool yield for each region and the entire fleece.

six hours to drive off the moisture. The bone-dry yields may be divided by 0.88 to obtain clean yields on a commercial basis which contain 12 per cent moisture.

Results

The clean wool yields from the 8 body regions are shown in table 1 and figure 1. Highest yields were obtained from the shoulder, thigh, withers and belly with yields of 39.8, 39.7, 39.6 and 39.6 per cent of clean wool respectively. Lowest yields were obtained from the rump, side and back with yields of 31.3, 31.8 and 33.5 per cent of clean wool respectively. No region gave a percentage yield which was very close to that of the entire fleece, (35.4%). It is possible that the same relationship in clean yield among the small samples may not obtain each year. The average yield of the samples from the 8 regions was 36.6 per cent which was somewhat higher than that for the entire fleece.

It is noted that higher yields of clean wool were obtained from the withers shoulder, belly, thigh and hip. Thus there appears to be a band of higher yielding wool extending around the outer edge of the shorn fleece from the withers down to the belly and around to the hip. Samples with lower yields were taken from the side, back and rump or the more central portion of the fleece.

TABLE 1.—MEAN YIELDS AND STANDARD DEVIATIONS OF SMALL SAMPLES AND WHOLE FLEECES AND CORRELATIONS AND REGRESSION COEFFICIENTS OF SMALL SAMPLES WITH WHOLE FLEECES, BASED ON 30 FLEECES

Region	Mean (per cent)	Standard deviation (per cent)	Correlation r	Regression coefficients	
				a	$b_{y.x}$ with standard error
Withers	39.6	4.7	.45	+17.47	.63± .238
Back	33.5	5.3	.61	− 0.50	.96± .239
Rump	31.3	3.7	.51	+11.12	.57± .181
Shoulder	39.8	4.1	.75	+ 7.54	.91± .152
Side	31.8	3.7	.69	+ 4.57	.77± .151
Hip	37.5	4.5	.54	+11.96	.72± .212
Thigh	39.7	3.0	.73	+14.75	.70± .124
Belly	39.6	3.0	.72	+16.59	.65± .118
Mean of 8 regions	36.6	2.9	.84	+10.45	.74± .091
Mean of back, shoulder and side	35.0	3.6	.81	+ 3.87	.88± .120
Whole fleeces	35.4	3.3			

Variations among regions and among individuals were highly significant as shown by analysis of variance in table 2. Furthermore the variation among regions was greater than that among individuals. This shows the importance of the regions from which the small sample is taken in estimating the percentage of clean wool in the entire fleece for the purpose of comparing individuals. Variability among individuals at the same region as shown by the standard deviations in table 1 was greatest at the back and lowest at the thigh and belly.

The relationship between the percentage of clean wool from small samples, taken from different regions of the fleece, and that from the entire fleece is shown by the means, correlation and regression coefficients in table 1. The highest association between the small samples and the entire fleece was ob-

tained with samples taken from the regions of the shoulder, thigh and belly as shown by correlation coefficients of 0.75, 0.73 and 0.72 respectively. Slightly lower correlations of 0.69 and 0.61 were obtained with the side and back respectively. The poorest association was found with samples taken from regions of the rump and withers with correlation coefficients of 0.51 and 0.45 respectively. However, all of the correlation coefficients with the exception of one were highly significant, exceeding the required value of 0.46. The correlations were not significantly different from each other. While the correlations between the small sample and whole fleece were high for the shoulder, thigh and belly in each case the yield determined from the small sample was higher than that from the whole fleece. This is not serious for breeding purposes as it will not interfere with comparisons of individuals or groups of individuals in the same flock. However, it is not desirable for

TABLE 2.—ANALYSIS OF VARIANCE OF PER CENT OF CLEAN YIELD

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Sheep	29	2001	69.0	7.5**
Regions	7	2969	424.1	46.1**
Discrepance (error)	203	1859	9.2	
Total	239	6829		

** Highly significant (>.01).

commercial purposes where the absolute clean yield is necessary. The average of samples from the back, side and shoulder gave a correlation coefficient of 0.81 with the entire fleece which was only slightly less than the coefficient of 0.84 obtained from an average of the 8 samples. Furthermore, the mean yield for samples from the back, side and shoulder of 35.0 per cent was very close to that of the entire fleece. It would appear that an average of three samples from the back, side and shoulder would be more representative of the entire fleece than any one sample from these regions. Furthermore the average of the samples from the back, side and shoulder would be as representative of the entire fleece as the average of the samples from all 8 regions.

The regression coefficients presented in table 1 may be used to estimate the clean yield of whole fleece (X) from the small sample value (Y) by use the following regression equation: $X = \frac{Y - {}^{a}y \cdot x}{{}^{b}y \cdot x}$.

It is noted that the regression (${}^{b}y \cdot x$) coefficients are all less than 1.0 while Schott *et al.* (1942) found that this coefficient for similar data was 1.11. The ${}^{b}y \cdot x$ regression coefficient would be expected to be about 1.0 if errors in

the clean yield determinations for the whole fleeces were at a minimum and were not correlated with errors in the clean yield determinations for the small samples. Inasmuch as the regression coefficients were consistently low it would be expected that the factors causing them to be low were operating throughout the data. These factors probably would not affect the regression coefficient for one region more than that for any other region. It is apparent from the above results for one year that per cent of clean yield obtained from a small sample of the shoulder, belly, thigh, side and possibly the back would be adequate for use in a breeding program where it would be used to compare individuals or groups of individuals within a flock. Here one would be interested in a value which would be comparable from one individual with another rather than one which would best estimate the absolute yield of clean wool for breed comparisons or marketing purposes. For the latter purposes in this study the clean yield of the shoulder, belly and thigh samples were higher and that of the back and side samples were lower than the clean yield from the entire fleeces. Further work is needed to determine whether a single sampling region could be found which would be more fully representative of the entire fleece than any of the samples taken in this study.

Summary

Clean wool yields of samples from 8 body regions, namely: withers, back, rump, shoulder, side, hip, thigh and belly were compared with one another and with the yield of the entire fleece to determine the variability of clean wool yield within the fleece and the relative reliability of samples from the various regions for predicting the clean wool yield of the entire fleece. The entire fleeces and 240 small samples were obtained from 30 Rambouillet yearling ewes during May 1939, at the Western Sheep Breeding Laboratory, Dubois, Idaho.

Highest clean yields were obtained from the shoulder, thigh, withers, and belly with yields of 39.8, 39.7, 39.6 and 39.6 per cent of clean wool respectively. Lowest yields were obtained from the rump, side and back with yields of 31.3, 31.8 and 33.5 per cent of clean wool respectively. The yield of clean wool for the entire fleece was 35.4 per cent. Variation among regions and among individuals were highly significant. Variation among regions was greater than among individuals.

Correlations between the small samples from the 8 regions and the entire fleece were not significantly different. The highest association between the small samples and the entire fleece were obtained from the regions of the shoulder, belly, thigh and side. Averages of samples from all 8 regions or from the shoulder, side and back were more representative of the entire fleece than samples from any one region.

Results for one year indicate that per cent of clean yield obtained from a small sample of the shoulder, belly, thigh, side and possibly the back would be adequate for use in a breeding program. Average yields of all 8 regions or of the shoulder, side and back regions were nearer to that of the entire fleece than any one region. Further work is underway to improve the sampling method for clean yield from individual sheep.

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FIBER DENSITY AND SOME METHODS OF ITS MEASUREMENT IN THE FLEECES OF RAMBOUILLET SHEEP

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Introduction

STUDIES of fiber density have been handicapped by a lack of knowledge of the accuracy of different techniques available for measuring density and the tremendous variations found within and between different regions of a fleece. The purpose of this study is to evaluate the accuracy of three density calipers and to investigate methods for controlling these large variations. Fiber density may be expressed in various ways. In the present study it is defined as the number of fibers per unit of skin area.

Calipers and other methods for measuring density have been described by Nordby (1928), Burns and Miller (1931), Hardy (1932), Wildman (1936), Burns (1937), Carter (1939), Hardy (1942), and Hardy and Wolf (1942). Some of these workers and Bosman (1934, 1937) have studied variations in density within fleeces of various breeds of sheep, but adequate data are not yet available to determine the relative merits of the various methods of sampling and to evaluate variations within fleeces.

Materials and Methods

Two groups of 10 sheep were selected at random from 104 thrifty yearling Rambouillet ewes at the Western Sheep Breeding Laboratory, and U. S. Sheep Experiment Station, Dubois, Idaho. Ten sheep in part 1 were used to furnish information on the variation in fiber density within a Rambouillet flock and on the accuracy of one caliper for measuring wool density. The remaining 10 sheep in part 2 were used for comparison of three wool density calipers. Samples[2] were taken from the following eight regions on each sheep: (1) withers, (2) back, (3) rump, (4) shoulder, (5) side, (6) hip, (7) thigh, and (8) belly, see figure 1. Three samples were taken at locations determined by chance and approximately four centimeters apart in each of the eight regions. The samples were analyzed in the wool laboratory at the Beltsville Research Center, Beltsville, Maryland. They were cleaned in carbon tetrachloride before being measured.

[1] The authors acknowledge assistance of R. H. Burns in obtaining samples in part 2 of the exepriment, of J. I. Hardy in various technical phases of the work including the construction of the Hairpin caliper, and D. A. Spencer, R. W. Phillips, J. E. Nordby, W. V. Lambert and R. G. Schott in the planning of the study and in interpreting results.

[2] The samples consisted of locks of wool removed from small skin areas of definite size. The terms lock and sample are used synonymously except where otherwise indicated.

Group 1 was sampled with the Hairpin wool density caliper (figure 2). The skin area demarcated measured 0.325 square centimeter. The locks removed from this area were cross-sectioned $\frac{3}{8}$ inch from the base end of the fiber using the technique described by Hardy (1935). Individual fibers were counted[3] and the number adjusted to density per square centimeter.

In part 2, three different calipers were used to secure the three samples from within each region. Sample A, or the first lock, was taken by the Hair-

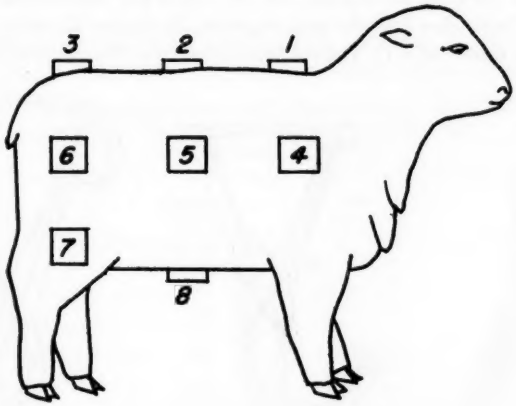

Figure 1. Regions sampled for fiber density.

pin caliper. Sample B was removed by the Wira caliper described by Wildman (1936), a mechanically improved Wyedesa caliper, and sample C was obtained with the Wyedesa caliper described by Burns (1937). In construction (figure 2), the Wira and Wyedesa calipers are very similar. Both were adjusted to demarcate areas of one square centimeter. The fiber density expressed as the number of fibers per square centimeter was determined by the cross-section-area method proposed by Hardy and Wolf (1942). The correlation between the number counted and the number estimated by the above method has been shown to be +0.995 or nearly perfect.

Experimental Results

Part 1

In part 1, the average density of the 10 sheep was 5,280 fibers per square centimeter. However, the density of individual sheep varied from 4,533 to 6,386 fibers. The order of density for the different regions was: withers

[3] Acknowledgment is made to Harold H. Spiegel, formerly with B.A.I., for doing much of the individual fiber counting.

(6,113), hip (6,011), rump (5,893), shoulder (5,742), side (5,611), back (4,983), thigh (4,105), and belly (3,784). The shoulder was the most variable and the and the hip the least variable. The mean density of 3 locks from individual regions ranged from 1,686 to 8,501 fibers. The average densities for the first, second, and third locks taken were 5,433, 5,202 and 5,205 respectively, while individually they varied from 424 to 10,163 fibers.

It was possible in an analysis of variance to differentiate between the sheep and between the regions, but one could not differentiate between the three locks taken from the same region on the basis of the order in which they were taken (table 1). The analysis showed that the differences between

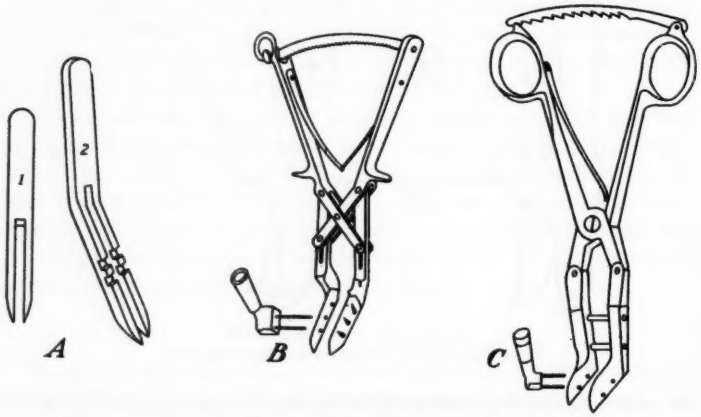

Figure 2. Three types of wool density calipers (A) Hairpin caliper consisting of parts 1 and 2 (B) Wira caliper (C) Wyedesa caliper.

regions on the same sheep were greater than the average differences between sheep. The fact that the variance between sheep and between regions were both significantly larger than the interaction between sheep and regions can be interpreted as meaning that there was a tendency for the differences between sheep to be discernible in a majority of the regions and for the differences between regions to be discernible in a majority of sheep. Each region analyzed separately showed that only for the withers and hip could one differentiate between sheep and that order of taking the samples (locks) was not significant. Three regions, namely the hip, rump and side were found to be correlated significantly in density with the mean of eight regions, see table 3. If only one lock was considered per region, it was no longer possible to differentiate between sheep. Also, the significance of the variance between sheep was reduced by disregarding the variation between locks.

The fact that the variation between the means of the locks was not significantly different from the variation due to the locks within regions and within sheep (table 1) indicates that the technique of taking samples was not biased. There was, however, a large uncontrolled variation between the locks taken from the same region and the same sheep as indicated by the range in samples within regions and the size of the standard deviation—1,506 fibers.

Part 2

The differences between the three calipers are shown in table 2 to be highly significant. There was no significant difference between means, 4,996

TABLE 1.—ANALYSIS OF VARIANCE FOR DENSITY OF FLEECE OF 10 RAMBOUILLET YEARLING EWES (FIBERS PER SQUARE CENTIMETER). PART 1

Source of variation	Degrees of freedom	Mean square	F
Sheep	9	7,790,227	3.43**[1]
Regions	7	24,141,101	10.64**[1]
Sheep×regions	63	3,065,533	1.35[1]
Locks within regions and within sheep	160	2,268,416	
Locks	2	1,403,382	1.72[2]
Sheep×locks	18	1,782,842	1.35[2]
Regions×locks	14	1,780,238	1.35[2]
Remainder: Sheep×regions×locks	126	2,405,757	
Total	239		

** Highly significant (the F value exceeds that necessary at the 1% level), see Snedecor (1940), table 10.3, pages 184–187).

[1] Variance between sheep was tested against the locks within regions and within sheep, since the variance from locks was not significant.

[2] Variance between locks was tested against the remainder or interaction sheep×region×locks.

and 4,979 fibers, determined by the Hairpin and Wyedesa calipers, respectively, but the value of 5,716 by the Wira calipers was significantly larger than the other two. However, the Wira and Wyedesa calipers ranked six of the 10 sheep similarly, while the Hairpin caliper ranked only one the same as did the other two. A highly significant correlation of 0.88 existed between the Wira and Wyedesa calipers.

Although it was possible with each caliper to differentiate between sheep and between regions, there was a difference in the accuracy of the calipers. If the sheep times regions interaction is considered to be the best estimate of the error, the Wyedesa caliper has the greatest accuracy. The accuracy with which the instruments sample the fiber density is illustrated in table 4.

TABLE 2.—ANALYSIS OF VARIANCE OF RESULTS FOR PART 2

Source of variation	Degrees of freedom	Mean square	F
Sheep	9	10,052,873	11.26**
Regions	7	40,665,304	45.58**
Calipers	2	14,195,675	15.91**
Sheep×regions	63	1,268,427	1.42*
Sheep×calipers	18	3,209,936	3.59*
Regions×calipers	14	1,235,919	1.39
Sheep×regions×calipers	126	892,086	
Total	239		

* Significant difference.
** Highly significant difference.

As in part 1 the eight body regions varied considerably in fiber density and in their ability to differentiate between sheep. The regions ranked as follows: withers (6,530), rump (6,336), hip (6,099), back (5,547), shoulder (5,347), side (4,863), thigh (3,622), and belly (3,502). The withers was ranked as the densest region and the side and back ranked in the same order by all three calipers. With the exception of the rump and shoulder regions, the remaining five regions did not differentiate between sheep. The correlations of individual regions with the combination of the eight are seen to differ for the three calipers (table 3). The rump was the only region for which these correlations were high for all calipers. The thigh and belly, with lowest correlations, were significantly less dense than the other positions.

TABLE 3.—CORRELATION OF EACH REGION WITH THE TOTAL[1]

Body Region	Correlation Coefficient				
	Part 1	Part 2			Parts 1 and 2 combined
		Hairpin	Wira	Wydesa	
Withers	0.523	0.588	0.675*	0.749*	0.314
Back	0.366	0.717*	0.295	0.588	0.626**
Rump	0.763*	0.796**	0.823**	0.874**	0.597**
Shoulder	0.304	0.810**	0.501	0.724*	0.566**
Side	0.688*	0.662*	−0.367	0.599	0.640**
Hip	0.852**	0.703*	0.797**	0.176	0.742**
Thigh	0.449	0.509	0.418	0.279	0.576**
Belly	−0.013	0.647*	0.587	−0.456	0.628**

[1] Groups in parts 1 and 2 combined made a total of 20 sheep. From each sheep the 8 regions were sampled by taking one lock with the Hairpin caliper.
* Significant for 8 degrees of freedom in parts 1 and 2.
** Highly significant for 8 degrees of freedom in parts 1 and 2, or 18 degrees of freedom in the combination of parts 1 and 2.

TABLE 4.—DIFFERENCE IN NUMBER OF FIBERS PER SQUARE CENTIMETER REQUIRED TO BE SIGNIFICANT[1]

Comparisons made	Part 1[2]	Part 2			
		Hairpin[3]	Wira[3]	Wyedesa[3]	Total[2]
Between sheep	923	1,349	1,225	1,102	569
Between regions	819	1,162	1,048	943	519
Between calipers	—	—	—	—	304
Between locks	495	—	—	—	—

Combinations of Parts 1 and 2 above[3]

	Numbers of sheep in each group				
	1	5	10	15	20
Between means of sheep in groups	1,567	600	517	339	293

[1] The above values were calculated using the formula $d = ts\sqrt{2/n}$ in which d = the necessary difference; t is taken from Snedecor (1940), page 58, Table 3.8; s is the best estimate of the standard deviation based on the variance left after all sources of significant variation have been removed; and n is the number of observations.

[2] 24 observations per sheep.

[3] 8 observations per sheep.

Discussion

A comparison of the two groups of 10 sheep showed them to be similar with regard to their mean density, 5,280 and 5,231 fibers being found respectively for groups in parts 1 and 2. The difference of 49 ± 212[4] fibers was clearly not significant. In both groups the withers was the densest region and the thigh and belly the least dense regions. The order of density of the other regions, however, differed in the two groups. A combination of the two groups, using only the first lock per region, reduced the uncontrolled error and made the variance between sheep and between regions more significant than when 10 sheep were considered.

The body regions contributed greatly to the variation found in both parts 1 and 2. However, certain regions were very similar in density despite their different anatomical locations. Three groups of regions which showed differences less than required for significance (table 4) were as follows: withers, rump, and hip (6,055 to 6,321 fibers); shoulder, side, and back (5,237 to 5,544); and thigh and belly (3,643 to 3,864 fibers). The rump, hip, and belly were selected as being representative of the variation throughout the fleece. A combination of the 20 sheep with one sample per region, lock A, from the three regions gave a rank correlation of 0.85 with the total of eight regions. This furnishes a better estimate than would be possible by using one region (table 3).

[4] Pooled standard error of the differences.

An important consideration in any breeding program is the magnitude of the difference required to discriminate between sheep and to distinguish between groups of offspring from different sires. Data in table 4 clearly indicate that the number of fibers to show significant differences will have to be quite large. The differences in the mean number of fibers per group required was found to range from 1,567 fibers for one sheep to 293 fibers with a group of 20 sheep, using the Hairpin caliper with the eight regions and one lock per region.

Part of the uncontrollable variation found in part 1 may be caused by density gradients or patterns within the regions, but since the locks were taken at random, this can not be determined. It is obvious that either a num-

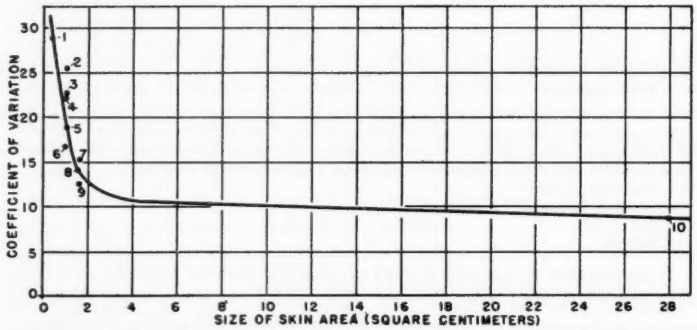

Figure 3. Curve showing the relation of the coefficient of variation within sheep and region to the size of the skin area from which each lock was taken. Numbers opposite each point indicate sources of data, as follows: 1 and 2—data from present study; 3 and 4—Wildman (1936a); 5 and 10—Madsen, *et al.* (1941); 6, 7, 8 and 9—Burns (1931, 1937).

ber of locks should be taken from each region or a fairly large sample should be taken to evaluate the density of the fleece in a given region. Increasing the number of locks taken from one to three per region increased the accuracy of determining the means both for sheep and for regions. Since the average standard error of the mean for a region was about 355 fibers, taking three locks by the Hairpin caliper from eight regions on 10 sheep, it would be necessary to take five locks to reduce the standard error below five per cent of the mean. In the present study, the only data on the effect of increasing the size of the area from which the locks were taken are the values in part 2 using the Hairpin caliper in which the area was 0.325 of a square centimeter and the other two calipers in which the area was one square centimeter. From results obtained in this and other studies, it appears probable that the relative variation of samples within a region decreases quite rapidly as the

area from which the sample is taken increases until a size of about 3 square centimeters is reached and then the variation becomes relatively constant (figure 3).

If the three calipers are all adjusted accurately and the jaws part the wool to give well-defined areas, no difference should result from design. The superiority of the Wira and Wyedesa over the Hairpin caliper may be attributed mainly to the larger skin area defined by these instruments. Both the Wira and Wyedesa instruments with the clamping feature make the sampling easier. Speed of sampling should be considered and proper credit given the faster instrument. The contour of the jaws of the calipers would have an important effect on the ease with which the wool is parted. Factors affecting the adjustment of the instrument other than design may be the precision of construction and the care in handling or operating. A slight alteration in the distance between the points of the caliper's jaws would make a noticeable difference in the mean values obtained. If the error is to be reduced to a minimum, the skin area sampled should have a fiber distribution pattern similar to that of the larger body region. The activity of the sheep and the position of the sheep during sampling have a definite effect on size of skin area.

Conclusions

The two groups of 10 Rambouillet yearling ewes which were studied were shown to be quite similar in mean fiber density and in the density of certain regions, but to vary for certain other regions.

Five locks per region would be necessary to reduce the standard error below five per cent of the mean.

Increasing the size of the skin area sampled increases the accuracy of determining fiber density at least until approximately three square centimeters have been reached.

Increasing the number of sheep sampled from 10 to 20 reduced the uncontrolled error and made the variance between sheep and between regions more significant.

The eight body regions fell into three groups of similar fiber density. Three regions, rump, hip, and belly, were highly correlated with the total of eight regions and were superior to any one region in the prediction of the fiber density.

The Wyedesa caliper appeared slightly more accurate than either the Wira or Hairpin calipers. Proper adjustment of the calipers and the size of the skin area sampled were probably the most important factors if the design of the jaws was correct.

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WOOL FINENESS IN EIGHT SAMPLING REGIONS ON YEARLING RAMBOUILLET EWES

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FINENESS and variability of wool have long been recognized among sheep and wool investigators as important factors in evaluating fleeces of sheep in a breeding program for wool improvement. These factors are fundamental in selecting sheep, in determining price of wool, and in the manufacturing industry.

Methods for determining fineness and variability have been previously studied and described by Duerden and Bosma (1926), von Bergen (1932; 1935; 1936), Hardy (1935), Burns (1935), Malan, Carter and Van Wyk (1938), Larose and Tweedie (1938), Hardy and Wolf (1939), Grandstaff (1940), Phillips, *et al.* (1940), and Pohle (1940). These efforts have been largely concerned with the development of methods for accurately evaluating fineness and variability in a single sample.

Wilson (1929) working with Romney rams observed differences in mean diameter between sheep and between different sampling regions on the same sheep. Pohle (1942) reported significant regression and correlation coefficients for fineness of fiber between weanling and yearling side samples but these were not significant for the thigh wool in Rambouillet sheep. He concluded that samples should be considered individually for the side and thigh so that any great variation between these areas on the fleece might be detected. Ensminger (1942) in a study to determine the variation in fiber diameter of wool samples secured from specific loci on Shropshire and Southdown sheep, found that no sharp line of demarcation can be drawn between fiber characters over adjacent areas.

The study reported here was designed to compare the variation in samples taken from different parts of the fleece in Rambouillet yearling ewes in order to determine what method of sampling would best represent the entire fleece. Emphasis has been placed on the following points:

(a) The variation in fiber diameter between different body regions.
(b) The fineness and extent of variation of fiber within specific regions of the fleece.
(c) The variation in fineness throughout the length of the fiber.
(d) The influence of the above factors on the accuracy of sampling individual fleeces.

[1] Acknowledgment is made to Dr. L. N. Hazel for his assistance and suggestions in the statistical analysis of this study.

Material and Methods

Two hundred and forty samples of wool were taken from ten Rambouillet ewes at the Western Sheep Breeding Laboratory, Dubois, Idaho. These sheep had been maintained under typical range conditions in the Intermountain West at an altitude ranging from 5,000 to 9,000 feet above sea level.

An effort was made to get representative animals of the flock for fineness of wool when judged by appearance from the side of each animal. Three judges who were accustomed to scoring sheep, estimated the fineness independently on each of 104 Rambouillet yearling ewes. The estimated spinning count for each ewe was taken as the average of the three estimates, and

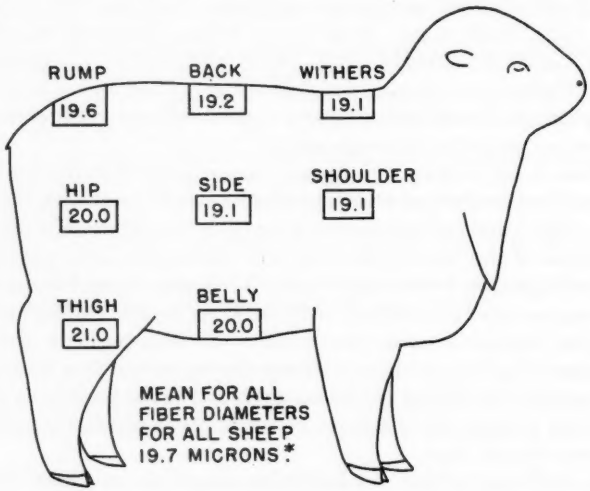

Figure 1. Regions sampled and the mean diameter in microns for each region. * Micron is 1/25,400-inch.

placed on a card. The 104 cards were divided into five classes according to the estimated spinning counts of wool which were 68's, 70's, 72's, 74's and 78's. The cards were then shuffled by classes and two cards drawn from each class to obtain the ten sheep used in the study.

Samples were taken in May 1939 from the right side of each sheep and from other regions on the body as illustrated in Figure 1:

Three samples (locks) were taken from each of the eight regions of each sheep. Each region was approximately eight centimeters square and the

three locks were taken four centimeters apart and so spaced that they fell at random within the region. A celluloid equilateral triangle measuring four centimeters was used for this purpose and the randomizing was based upon the use of 12 metal discs numbered individually from one to twelve. These discs were shuffled to assist in randomizing the selection of the three sub-locations in each of the eight regions in each fleece. At each shuffling of the metal discs the number was read on the disc subsequently drawn and the triangle set on the area pointing to the corresponding location on the dial of a clock, the other two points of the triangle falling where they would. The three locks from each region were taken at the points of the triangle.

A sample of wool approximately 2-3 millimeters in diameter was clipped close to the skin with a pair of curved dissecting scissors and handled so as to prevent disturbance of the fibers in each lock. The locks were carefully placed in coin envelopes that were properly labeled to show the number of the ewe, type of sample (fineness), region of the fleece, sub-location of the point within the region as lock (A), (B) or (C), in the order of sampling, and the date of taking the sample.

All samples were cleaned in carbon tetrachloride to remove grease and dirt. Photostatic cross-sections as described by Hardy (1935), magnified 500 diameters, were made at three levels on each lock, 15 mm. from the base, at the middle, and 15 mm. from the tip. Fineness and uniformity on each cross-section were determined by individual bi-diameter fiber measurements four groups of 50 fibers, making a total of 200 fibers for each of the three locations along the fiber. The four groups of 50 fibers each were taken on the photostats by measuring all 50 fibers adjacent to each other until the first 50 were measured and recorded, and then another group of 50 was counted that was adjacent to the first block, and this procedure continued until a total of 200 individual fibers were measured. All of the foregoing operations were carried out at the Western Sheep Breeding Laboratory and United States Sheep Experiment Station, Dubois, Idaho.

All mean diameters were obtained in this study by measuring individual fibers in order to have greater accuracy of results. However, in the practical determination of wool fiber fineness very satisfactory results may be obtained by the rapid comparator method described by Pohle (1940).

Results

A general picture of the sampling regions in relation to the whole population of fibers and the distribution of the averages for the individual sheep is given in figures 1 and 2. The average diameters for each region are shown in table 1.

The distribution of the means of sheep by regions shows that the dif-

TABLE 1.—MEAN DIAMETERS IN MICRONS FOR EACH SHEEP BY REGIONS BASED ON 200 FIBER MEASUREMENTS FROM EACH OF THE BASE, MIDDLE AND TIP ON THREE LOCKS OF WOOL (1800 MEASUREMENTS FROM EACH REGION)

Sheep No.	Regions								Sheep Av.
	Withers	Back	Rump	Shoulder	Side	Hip	Thigh	Belly	
	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)
2120	16.5	16.9	17.2	17.0	16.1	17.6	18.9	16.9	17.1
149	18.3	18.0	17.8	17.6	17.5	18.5	20.0	18.5	18.3
99	18.5	18.0	19.0	17.7	17.9	19 9	19.6	18.4	18.6
2566	19.9	19.3	19.8	19.1	19.0	19.9	21.3	20.6	19.9
2338	19.4	19.2	20.0	19.1	20.1	20.9	21.2	20.3	20.0
3910	18.5	19.0	19.7	19.9	19.9	21.0	22.1	20.8	20.1
151	19.2	19.4	19.9	19.7	19.7	21.0	21.2	22.0	20.3
2347	20.7	20.1	20.9	20.8	19.7	20.5	20.4	19.4	20.3
3897	19.6	20.5	20.4	20.0	21.3	19.9	21.9	21.4	20.6
2397	20.6	21.0	21.7	20.7	21.0	21.4	23.4	21.7	21.4
Av. all Regions	19.1	19.2	19.6	19.1	19.1	20.0	21.0	20.0	19.7

ferences between individual sheep are fairly consistent for different regions. There appears to be a trend in average fineness from the smallest average diameter at the withers, shoulder, back and side, to larger average diameters at the rump, hip and belly, to the coarsest fibers at the thigh.

Table 2 shows the analysis of variance of the 144,000 fiber diameters.

TABLE 2.—ANALYSIS OF VARIANCE FOR 144,000 FIBER DIAMETERS

Source of Variance	Degrees of Freedom	Mean Squares	F*	Component of Variance
Total	144399	15.57		
Sheep	9	23500.46	22.68**	$\sigma_s^2 = 1.5349$
Regions	7	7967.12	8.44**	
Levels	2	25127.82	23.65**	
Sheep×regions	63	581.28	2.38**	$\sigma_{sr}^2 = 0.2527$
Sheep×levels	18	699.61	2.86**	$\sigma_{sl}^2 = 0.0701$
Regions×levels	14	607.38	2.48**	
Sheep×regions×levels	126	244.72	19.16**	$\sigma_{srl}^2 = 0.3866$
Remainder	143760	12.77		$\sigma_e^2 = 12.77$

* Since this experimental design does not yield directly valid estimates of error for testing the three main sources of variation, the error term used in testing the significance of each main source was the sum of the two-factor interactions involving that source minus the triple interaction.

** Significant at 1% level.

The average fiber diameters for the three locks taken in each region were 19.75, 19.61, and 19.61 microns respectively for locks A, B and C. The first lock taken was designated as A, the second as B and the third as C. The original analysis was accomplished by keeping the three locks distinct, but, since the differences between locks and the interactions between locks and the other major classifications might have been due to random fluctuations, the mean squares for the sources of variation were not presented in the analysis. Fairbanks (1931) concluded "when duplicate samples are taken one inch apart and from rather small areas there are significant differences between the arithmetic means of their diameters." Burns (1931) working on fleece analysis in sheep reported the "variation between mean micrometer fineness of quadruplicate samples contiguous to a selected point on the body was negligible." These results support the unbiased nature of the sampling and measurement technique. Differences between sheep were highly significant. Since differences between regions were also significant, this suggests that the gradient previously observed in the fleece (figure 2) is real. These results are in accordance with those found by Burns (1931). Differences between levels were also highly significant. The average diameters at the base, middle and tip were 19.40, 19.10 and 20.47 microns, respectively. Differences in levels may be due to age changes and to environmental differences due to feed and climatic conditions at the different seasons when the portion of the fiber sectioned was grown. The tip portion was grown before weaning when the fiber diameter is generally larger for Rambouillets.

Even though a distinct gradient was evident in the fleeces of the ten sheep, the significance of the sheep×regions interaction indicates that individual sheep deviate from the general gradient, being either finer or coarser in particular regions than is indicated from their average diameter and from the averages for those regions. Bailey (1941) also observed a significant interaction for sheep×regions for the shoulder, middle and thigh regions. The effect of this interaction upon the 80 sheep-region means is illustrated graphically in figure 2. A part of this variation may be due to the sampling technique wherein the three locks were taken at random within the limits of a relatively large region. Naturally the precise location from which the locks were taken varied slightly from one sheep to another. However, the interaction of sheep×regions, as far as can be determined from these data, is a part of the natural variation observed in fleeces and consequently must be considered in sampling when estimating average diameter.

The sheep×levels interaction measures the extent of the differential results from sampling sheep at different levels. Since this interaction is significant, it suggests that sheep appear either finer or coarser at some levels than would be supposed from their average diameter. This may represent actual peculiarities in the fiber-growth pattern of individual sheep or it may

represent an artificial situation incurred by the sampling technique. Fibers were cut and their diameters measured 15 mm. from the tip, at the middle and 15 mm. from the base of the fiber. Since these yearling ewes were born over a period of 60 days, fibers from a given level on different sheep were

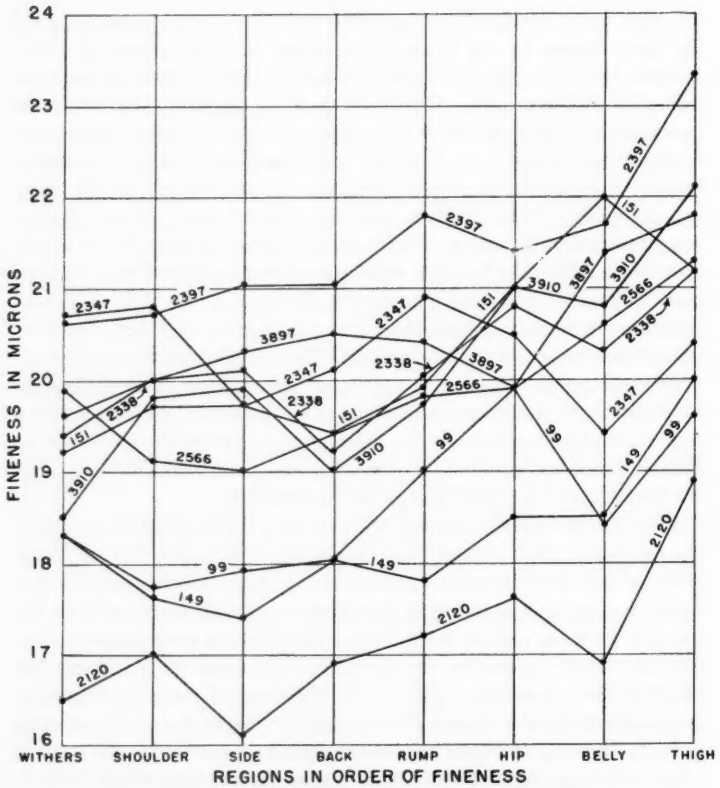

Figure 2. Showing average fineness for level of the total and distribution of averages for sheep and for regions.

measured at points which grew at different times. If general environment, such as feed conditions, winter storms, trailing, etc., are responsible for the sheep × levels interaction, this source of variation might be negligible if fibers could be measured at points grown at the same time. If fibers follow a growth pattern influenced by age, the better plan would be to measure fibers at levels which were grown at the same age for all sheep.

Evidence is not available as to the exact nature of the sheep×level interaction. Since it seems difficult, if at all possible, to control by experimental technique, it is regarded as a part of the natural variation to be considered in sampling fleeces.

The regions×levels interaction, although significant, is of no practical interest since it does not influence the accuracy of measurements for individual sheep. Its nature may be similar to that of the sheep×levels interaction because the wool in all regions on the same sheep does not increase in length at the same rate.

The triple interaction in table 2 is significant at the 1% level. This indicates that sampling different regions and different levels gives results unlike those obtained from sampling different regions alone or different levels alone. All or any of the factors suggested as being responsible for the two-factor interactions may operate together to produce this source of variation. In view of the obscure (but real) nature of this interaction, it must be considered a part of the natural variation.

The remainder in table 2 represents the variation in fiber diameters within a given lock plus any additional error incurred by sampling and measurement technique. Hence it is a measure of the minimum error beyond which accuracy could not be increased by sampling technique.

The variation associated with average differences between regions and between levels can be controlled by experimental design by measuring all sheep at the same regions and same levels where the object is to compare sheep for average diameter. However, this technique does not control the uncertainty attached to a particular region (sheep×regions interaction), that attached to any particular level (sheep×levels interaction) or that attached to any region at a given level (sheep×regions×levels interaction). Consequently, for comparing sheep sampled at the *same regions and levels*, the standard error of the mean of any animal is

$$s = \sqrt{\frac{\sigma_e^2 + k\sigma^2_{srl} + kl\sigma^2_{sr} + kr\sigma^2_{sl}}{krl}}$$

where k, r and l represent the number of fibers measured per lock, the number of regions and number of levels sampled, respectively. The components of variance (σ^2), associated with the sources of variation discussed above, were estimated from the analysis in table 2 and are given in the last column of that table. Winsor and Clarke (1940) presented the mathematical justification and examples of the method of estimation.

Table 3 gives the standard error, calculated by the above formula, expected for different values of k, r and l. The standard error decreases only slightly from $k = 100$ to $k = 600$, even for small values of r and l. In practice,

it is convenient to make photomicrographs of cross-sections of wool fibers which contain from 100 to 200 fibers depending upon the average diameter of the fibers and the closeness with which the fibers are packed into the cross-sectioning device. The slight effect which the increasing of k beyond 100 or 200 has in decreasing the standard error indicates that counting the fibers from more than one cross-section at a particular region and level is not efficient sampling technique. Malan *et al.* (1938) concluded from a study of the variability of composite wool samples that measuring 250 fiber diameters on a well-prepared slide provided accuracy sufficient for most purposes. Increased accuracy can most efficiently be obtained by increasing first the

TABLE 3.—STANDARD ERROR OF MEAN DIAMETER FOR INDIVIDUAL SHEEP FOR DIFFERENT VALUES OF k, r AND l

No. of levels (l)	No. of fibers (k)	No. of regions (r)				
		1	2	3	4	8
		Microns	*Microns*	*Microns*	*Microns*	*Microns*
1	50	.982	.719	.607	.542	.426
	100	.915	.673	.571	.512	.407
	200	.879	.649	.552	.496	.397
	400	.861	.637	.542	.488	.392
	600	.855	.633	.539	.485	.391
2	50	.780	.567	.476	.422	.327
	100	.738	.539	.453	.403	.314
	200	.716	.523	.441	.393	.308
	400	.705	.516	.435	.388	.305
	600	.701	.513	.433	.386	.303
3	50	.700	.507	.423	.374	.286
	100	.669	.485	.406	.360	.276
	200	.653	.474	.397	.352	.271
	400	.645	.469	.393	.348	.269
	600	.642	.467	.391	.347	.268

number of regions sampled and then the number of levels. Each additional level sampled requires an additional photostatic cross-section, while each additional region requires that a new sample be cut and prepared.

The sampling accuracy desired should be commensurate with the object for which measurements are made. For example, in sampling an extremely variable population, less accuracy is required to differentiate between fine and coarse fleeces than is required in an extremely uniform population. According to the American Society for Testing Materials (1941) wool top standards for fine wools (80's, 70's and 64's), the range from finest to coarsest fine wool fleeces is 4.5 microns. A standard error of 0.692 derived from $\left(\frac{4.5}{6.5}\right)$

would be consistent for 6.5 standard deviations within the range of a normally distributed population. According to table 3, samples from two levels and one region or preferably from one levels and two regions would be required to obtain a standard error this small. This degree of accuracy might be used in culling a band of ewes where the mean diameters extended over the entire range of fine wools. For an extremely uniform group such as exists within a single fineness grade (with a range of 1.5 microns), a standard error of 0.231 microns would be consistent with the range. According to table 3, this degree of accuracy could be obtained only by sampling more than eight regions and three levels.

The problem often arises in practice of comparing the wool of two sheep

TABLE 4.—MEAN DIFFERENCES BETWEEN SHEEP REQUIRED FOR 5% LEVEL OF SIGNIFICANCE FOR DIFFERENT VALUES OF k, r AND l

No. of levels (l)	No. of fibers (k)	No. of regions (r)			
		2	3	4	8
		Microns	*Microns*	*Microns*	*Microns*
1	50	12.92	3.69	2.44	1.42
	100	12.09	3.47	2.30	1.36
	200	11.66	3.36	2.23	1.33
2	50	10.19	2.90	1.90	1.07
	100	9.69	2.76	1.81	1.02
	200	9.40	2.68	1.77	1.00
3	50	9.11	2.57	1.68	0.93
	100	8.71	2.47	1.62	0.90
	200	8.52	2.42	1.58	0.88

in average diameter, and of being *reasonably sure* that a real difference exists. For example, one may wish to select a sire from a group of two or more rams on the basis of fineness. This problem can be answered by Student's t-test. In this case, it seems logical to base the t value for the comparison upon one less than the number of regions sampled since the estimate of σ^2_{sr} is considerably larger than that of σ^2_{sl}. The minimum differences required for the 5% level of significance for different values of k, r and l are given in table 4. These differences were calculated by the formula

$$d = ts\sqrt{2}$$

where s is the standard error for a particular value of k, r and l given in table 3 and t is taken for $r-1$ degrees of freedom.

The mean differences required for significance emphasize the importance of sampling several regions for accurate differentiation between sheep be-

cause each additional region sampled adds to the degrees of freedom upon which the standard error is based, while t decreases quite rapidly as the number in small samples increases. Bailey (1941) likewise found that fiber diameter should be measured at more than one point on the fleece.

The problem of comparing groups of sheep, such as progeny by different sires, inbred lines, etc., also arises in practice. While this study supplies no data on the subject, we have no evidence yet that interactions would exist between such groups and regions or levels. If these interactions do not exist, the standard error of the mean of each group is

$$s = \sqrt{\frac{\sigma_e^2 + k\sigma^2_{srl} + kl\sigma^2_{sr} + k\tau\sigma^2_{sl} + k\tau l\sigma^2_s}{k\tau ln}},$$

when n is the number of sheep per group. The estimate of the component of variance attritubed to real differences between sheep (σ^2_s in table 1) may be

TABLE 5.—MEAN DIFFERENCES BETWEEN GROUPS OF SHEEP REQUIRED FOR 5% LEVEL OF SIGNIFICANCE FOR DIFFERENT VALUES OF n, r AND l AND FOR $k=100$

No. of regions (r) and levels (l)	No. of sheep per group (n)					
	2	3	5	10	20	50
	Microns	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*
$r=1$, $l=1$	4.69	2.47	1.59	1.02	0.70	0.43
$r=2$, $l=1$	4.29	2.26	1.45	0.94	0.64	0.40
$r=4$, $l=2$	3.96	2.09	1.34	0.86	0.59	0.37

slightly larger in these data than would actually exist in a normally distributed population. In sampling the 104 sheep, two ewes from each of the five estimated fineness grades were included in the sample of ten. It seems unlikely however that this procedure could have affected the estimates of the other components of variance. The minimum differences required for the 5% level of significance for different values of n, r and l and for $k=100$ were calculated, t being taken for $2(n-1)$ as the correct number of degrees of freedom. These minimum differences (given in table 5) show that at least ten sheep per group are required to make a difference of one micron significant for a reasonable number of regions and levels.

It was found that samples of wool from the shoulder, side and thigh ranked the sheep in approximately the same order as samples from the 8 regions giving about the same average diameter. These positions are convenient to sample and provide an estimate of the variation in diameter for individual animals. It would seem therefore, that samples be taken from some combination of these regions.

Summary

Analyses are presented for fiber diameters of wool obtained from ten Rambouillet yearling ewes, sampled from eight body regions: withers, back, rump, shoulder, side, hip, thigh and belly. Three locks of wool from each region were taken, and each lock was cross-sectioned at three levels: near the base, at the middle and near the tip. Four groups of 50 adjacent fiber diameters were measured in each cross-section, making a total of 14,400 fiber diameters for each sheep or 144,000 for all animals.

There appears to be a trend in average fineness from the smallest average diameter at the withers, shoulder, back and side, to larger average diameters at the rump and belly, to the coarsest fibers at the thigh.

Differences between sheep, regions, locks, cross-sections and groups were examined to determine the minimum number of locations and number of fibers that would adequately characterize a fleece and so describe a working index for selection of experimental breeding sheep.

An increase in the number of fibers measured beyond 100 to 200 decreases the standard error but slightly, which indicates that measuring the fibers from more than one cross-section at a particular region and level is not efficient sampling technique. Increased accuracy can most efficiently be obtained by increasing first the number of regions sampled and then the number of levels along the fiber.

Samples from one level and two regions are required to obtain a standard error of 0.692 which is consistent with the range usually observed in fine wool sheep.

It was found that samples from the shoulder, side and thigh rank sheep in approximately the same order as samples from the eight regions.

For comparing individual fleeces several regions and levels must be sampled to make differences of from two to three microns significant at the 5% level.

In comparing fleeces from groups of sheep, such as progeny by different sires, results show that at least ten sheep per group are required to make a difference of one micron significant for a reasonable number of regions and levels.

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THE VALUE OF ALFALFA AND CERTAIN OF ITS FRACTIONS IN THE NUTRITION OF BREEDING EWES[1]

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IN A previous publication (Shrewsbury, *et al.*, 1942) we reported that a ration for breeding ewes composed of corn, corn silage and oat straw was deficient in protein and probably also in vitamin A. In addition we demonstrated that when alfalfa hay was fed in place of oat straw in the above ration, maintenance and lactation were satisfactory and lambs of good quality were produced. The present report covers a study of the amount of alfalfa hay required by ewes and the nutritive properties of various fractions of alfalfa. In addition preliminary observations are described on the value of dehydrated oat grass in the ration of breeding ewes.

Experimental

Fourteen lots of 15 yearling ewes each quartered as previously described (Shrewsbury, *et al.*, 1942) received the rations listed in table 1. The ewes were bred during October and November and were fed legume hay until they were placed on the experimental rations December 20, 1941. Special care was exercised in assigning the ewes to the different lots by random selection from 15 groups arrayed according to weight classification.

It was the plan to feed the roughages according to a predetermined schedule but considerable difficulty was experienced during the first few weeks of the experiment in effecting consumption, because of the fineness of the materials. For this reason average daily consumption of roughages covering the entire experiment was somewhat below the original estimates in some cases. It was also the plan to adjust the roughage consumption of all lots to 3 pounds per head per ewe per day by the use of oat straw but refusals of feed eventually caused us to abandon this procedure. The method finally adopted was to mix cane molasses with the roughage at a level of 20 per cent and to feed this separately from the other constituents of the ration. The carbohydrate content of the rations was partially adjusted with yellow corn.

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TABLE 1.—FEED CONSUMPTION OF EWES ON VARIOUS ROUGHAGES.
AVERAGE DAILY FEED IN POUNDS

Lot No.	Yellow corn	Corn silage	Dehydrated alfalfa hay meal	Dehydrated oat grass meal	Oat straw meal	Field cured alfalfa hay meal	Pressed juice of alfalfa[a]	Pressed juice of alfalfa[b]	Alfalfa fat solubles[c]	Alfalfa oil cake[d]	Cottonseed meal	Commercial casein	Ground limestone	Cane molasses	Total feed	Daily total protein per ewe	Daily energy therms per ewe[e]
1	0.43	1.0	2.48	—	—	—	—	—	—	—	—	—	—	0.60	4.51	0.53	1.92
2	0.93	1.0	1.61	—	0.10	—	—	—	—	—	—	—	—	0.40	4.04	0.42	1.86
3	1.20	1.0	0.86	—	0.17	—	—	—	—	—	—	—	—	0.23	3.46	0.30	1.69
4	1.30	1.0	0.42	—	0.54	—	—	—	—	—	—	—	—	0.21	3.47	0.25	1.66
5	0.66	1.0	—	2.21	—	—	—	—	—	—	—	—	—	0.53	4.40	0.65	1.95
6	1.37	1.0	—	—	0.70	—	—	—	—	—	—	—	—	0.16	3.23	0.18	1.55
7	0.94	1.0	—	—	—	1.9	—	—	—	—	—	—	—	0.43	4.27	0.45	1.98
8	1.58	1.0	—	—	0.94	—	0.41	—	—	—	0.29	—	0.13	0.23	4.58	0.30	2.24
9	0.85	1.0	—	—	0.14	—	—	1.75	—	—	—	—	—	0.44	4.18	0.38	1.84
10	1.41	1.0	—	—	0.95	—	—	—	0.18	—	0.30	—	0.09	0.22	4.15	0.32	2.16
11	0.81	1.0	—	—	0.13	—	—	—	—	1.67	—	—	—	0.42	4.13	0.38	1.76
12	0.66	1.0	—	—	0.12	—	0.44	1.16	—	0.06	0.42	—	—	0.39	4.25	0.34	1.96
13	1.28	1.0	—	—	1.00	—	—	—	—	—	0.34	—	—	0.24	3.86	0.33	1.75
14	1.17	1.0	—	—	1.00	—	—	—	—	—	—	0.46	—	0.23	3.86	0.54	1.85

[a] The pressed juice of fresh alfalfa concentrated to thick consistency.
[b] The residue of alfalfa after expression of the juice.
[c] Fat extractives of pressed cake of alfalfa.
[d] The residue of alfalfa after extraction of the pressed cake with fat solvent.
[e] Approximate.

Body weights of the ewes were obtained at the beginning of the experiment, at the end of pregnancy, and at the end of lactation.

The lambs were weighed at birth and at weekly intervals for the first 8 weeks following parturition. The milk production of each ewe was determined for a 12 hour period once each week for the first 8 weeks of lactation. This was accomplished (as fully described by the authors in 1942) by separating the lambs from their dams for 3 hour intervals, weighing the lambs to the nearest ounce, allowing them to suckle, and immediately reweighing and separating them for another 3 hour period.

All of the roughage materials and alfalfa fractions were supplied through an arrangement with Cerophyl Laboratories.[4] The materials were grown at approximately the same season of the year on comparable soil types. The dry

TABLE 2.—CHEMICAL ANALYSIS OF FEEDS[a]

Feed	Solids	Protein	Ash	Fiber	Carotene	Ascorbic acid	Riboflavin	Nicotinic acid	Pantothentic	Folic acid
	per cent	*per cent*	*per cent*	*per cent*	*mgms 100 gms*	*mgms 100 gm*	*mgm per gm*	*mgm per gm*	*mgm per gm*	*mgm per gm*
Dehydrated oat grass	91.5	25.6	9.1	18.2	45.8	312.5	22.9	71.0	8.7	0.7
Dehydrated alfalfa	92.6	18.6	12.0	26.4	15.5	56.3	15.1	45.0	15.8	0.9
Field cured alfalfa	88.1	17.7	7.7	25.2	0.3	18.8	7.0	36.0	13.9	4.0
Oat straw	88.7	5.4	6.5	37.3	0.5	18.8	5.7	16.0	7.6	2.9
Alfalfa press cake	90.2	15.2	8.6	32.2	2.0	12.5	13.4	42.0	13.2	1.3
Alfalfa oil cake	91.3	16.5	9.8	31.1	0.8	31.2	13.7	31.0	13.6	1.8
Alfalfa press juice	58.7	11.6	9.0	0.0	0.0	101.6	40.4	131.0	8.3	9.4
Alfalfa fat solubles					366.2					

[a] Analysis made by Cerophyl Laboratories, Kansas City, Missouri.

products were ground through a hammer mill and shipped in bags. The water and fat soluble fractions were kept under refrigeration until used. The analyses of the materials used are given in table 2.

The dehydrated alfalfa hay meal was prepared by rapidly processing the fresh mature alfalfa in an Arnold drier type dehydrator. The dehydrated material was then finely ground and bagged in burlap sacks. The dehydrated oat grass meal was prepared from oat plants 6 to 8 inches in height before jointing had taken place. The freshly harvested grass was dehydrated, ground and bagged similar to the alfalfa. The field cured alfalfa hay meal was

[4] The cooperation of the Cerophyl Laboratories, Kansas City, Missouri, in furnishing the roughages and fractions is gratefully acknowledged. Special thanks are due Doctor W. R. Graham for his interest and helpful suggestions in the experiment.

a third cutting, was sun cured in the field without rain damage and after curing was finely ground and bagged.

The fractionation of the alfalfa hay was accomplished by macerating the freshly cut hay and pressing out the juice with a screw press. The expressed juice was concentrated in vacuo at low temperature to a thick consistency. The alfalfa residue remaining after expression of the juice was designated pressed cake of alfalfa. A portion of this pressed cake was extracted with petroleum solvent and the residue dried at low temperature to remove the solvent. The solvent was evaporated from the extract by vacuum distillation at low temperature. These products were designated alfalfa fat solubles and alfalfa oil cake. The alfalfa oil cake and pressed cakes were finely ground and bagged.

All other feeds used in the experiment were purchased on the local market. Table 1 shows the average daily feed, the total feed, the average daily protein intake and the average daily energy content of the rations. The protein and energy calculations were made from analysis of the special feeds used and from Morrison's (1938) tables. In calculating the energy values for the special feeds the figures given for good quality alfalfa hay were employed since actual values were not available. This gave only an approximation but it was believed to be of sufficient accuracy for comparative value. Morrison's (1938) standards specify that breeding ewes of 100 pounds weight require about 2 pounds of dry matter and from 1.2 to 1.5 therms daily during the first part of pregnancy and about 2.5 pounds of dry matter and from 1.6 to 1.9 therms daily during the latter part of pregnancy. Reference to table 1 shows that, in general the rations used covered the requirements with the exception of Nos. 3, 4, 6. Rations 3 and 4 contained limited amounts of alfalfa hay as roughage by design and ration 6 contained oat straw in amounts greater than were actually consumed.

Table 2 gives the analysis of the special feeds. It will be seen that there is a large difference in the protein content of the dehydrated oat grass and oat straw. It is probable that there are also differences in the quality of the protein in the two materials. Dehydrated alfalfa and sun cured alfalfa contained a good quantity of protein but in lesser amount that the dehydrated oat grass. The oat grass also contained less fiber, more carotene, ascorbic acid, riboflavin and nicotinic acid than the other feeding materials. The very high ascorbic acid content of oat grass is of particular interest although its biological value is at present unknown. The field cured alfalfa hay contained a very small amount of carotene but a relatively high concentration of nicotinic acid. The possible significance of these findings will become apparent after consideration of the data that follow.

Results

Table 3 gives the data on the gains of the ewes during the period of pregnancy. One of the objects of the experiments was to define the amount of alfalfa hay required to maintain the ewes in a satisfactory condition during pregnancy. It was the plan to feed 3, 2, 1, and 0.5 pounds of hay per ewe per day in lots, 1, 2, 3, and 4 respectively but for reasons previously mentioned 2.48, 1.61, 0.86 and 0.42 pounds per head were actually consumed. In addition to hay all lots consumed 1 pound of corn silage per head daily and

TABLE 3.—THE MAINTENANCE DURING PREGNANCY OF EWES FED DIFFERENT ROUGHAGES[a]

Lot No.	Ration[b]	Average starting weight 12/20/41	Average weight at end of pregnancy 3/10/42	Average gain during pregnancy	Total average gain per ewe[c]	Difference in gain
		pounds	*pounds*	*pounds*	*pounds*	*pounds*
6	Oat straw	112.5	123.8	11.3	11.5	8.1
14	Casein	113.7	136.1	22.4	19.6	1.6
13	Cottonseed meal	113.1	137.5	24.4	21.1	0.4
4	Dehydrated alfalfa	113.4	135.8	22.4	21.5	5.7
10	Alfalfa fat solubles	112.8	142.5	29.7	27.2	0.0
11	Alfalfa oil cake	113.0	141.2	28.2	27.2	0.2
9	Alfalfa press cake	113.5	142.8	29.3	27.4	1.6
2	Dehydrated alfalfa	113.8	145.1	31.3	29.0	1.4
3	Dehydrated alfalfa	113.2	144.5	31.3	30.4	0.5
12	Recombined alfalfa	113.2	147.0	33.8	30.9	0.4
8	Alfalfa press juice	113.4	144.8	31.4	31.3	1.3
7	Field cured alfalfa	114.1	148.1	34.0	32.6	5.9
1	Dehydrated alfalfa	113.5	151.2	37.7	38.5	12.4
5	Dehydrated oat grass	114.1	168.3	54.2	50.9	

[a] 15 ewes started in each lot. Average weight 113.3 pounds. Period of observation Dec. 20, 1941, to March 20, 1942. Later reduced to 13 to conserve feed supply.

[b] See table 1 for composition of rations.

[c] Least significant mean difference in gain by analysis of variance = 12.5 pounds.

various amounts of ground yellow corn. The amount of corn was adjusted to equalize the energy content of the rations. The best results, as judged by the gain in weight, were obtained in lot 1 (38.5 pounds per ewe) where the greatest consumption of the roughage was effected and the slowest growth was in lot 4 (21.5 pounds per ewe) where the smallest amount of hay was consumed. Lots 2 and 3 which received intermediate amounts of alfalfa hay gained about the same (29.0 and 30.4 pounds) but these were somewhat less than the gains in lot 1.

When the data in table 3 were treated by the method of analysis of variance highly significant differences were found between lot mean gains; the

least significant mean difference was 12.5 pounds. The difference between the lots receiving the smallest and largest amounts of dehydrated alfalfa is highly significant, while differences between lot gains in this series approached significance in some cases (lots 1 and 2, lots 3 and 4).

Lot 6 received the ration containing oat st .w as roughage. This ration was previously found to be very deficient f breeding ewes (Shrewsbury, *et al.*, 1942). The ewes in this lot gained only 11.5 pounds as compared to a gain of 38.5 pounds for the ewes on dehydrated alfalfa (lot 1). Lot 7 ewes were fed field cured alfalfa and gained 32.6 pounds per head which compares favorably with the results obtained by feeding dehydrated alfalfa.

The most striking result was obtained by feeding dehydrated oat grass (lot 5). The ewes in this lot gained 50.9 pounds per head and were in excellent condition at lambing time. The ewes in the dehydrated and field cured alfalfa hay lots were in good condition throughout this period of the experiment; the ewes in the oat straw lot showed much less vigor.

An attempt was made to improve the nutritive properties of the oat straw ration by the addition of protein as cottonseed meal and casein (lots 13 and 14). While growth was considerably improved (21.1 and 19.6 pounds per ewe as compared to 11.3 pounds for the oat straw lot) the results were not comparable to those obtained with alfalfa hay or oat grass. The differences in gain approached statistical significance.

Since the proteins used are of good quality and since rather large amounts were consumed (about ⅛ and ½ pounds per head daily, cottonseed and casein respectively) it is concluded that protein, per se is not the sole limiting factor in the poor nutritive value of the oat straw ration. The data in table 3 and previous work (1942) show that both dehydrated and field cured alfalfa hay supplement corn and silage in a fairly adequate manner in the ration of breeding ewes. It was accordingly of interest to try to isolate the active potency in some fraction of the alfalfa plant. The results obtained are described in table 3 (rations 8 to 12). In rations 8 and 10, when alfalfa pressed juice and fat solubles respectively were fed, limestone was added in amounts approximately equal to the calcium contained in 3 pounds of alfalfa hay, since it was recognized that one of the deficiencies of the basal oat straw ration is calcium. All of the fractions tested proved to have definite nutritive value. This might indicate incomplete fractionation of important biological factors whose nature is as yet unknown during the fractionating process. The processing in itself did not seem to reduce materially the nutritive value of the alfalfa since the recombined fractions (pressed juice, fat solubles, pressed cake and oil cake) produced growth in the ewes in lot 12 (30.4 pounds) comparable to the untreated alfalfa lot 1 (38.5 pounds) and lot 7 (32.6 pounds).

Of particular interest are the results obtained in lot 8 where the alfalfa pressed juice was fed. The growth produced in this lot was only slightly less than that where field cured hay was used (31.3 pounds vs. 32.6 pounds per head). The ration fed in this lot contained added cottonseed meal and limestone.

It is interesting to note that ration 13 which also contained cottonseed meal produced gains of only 21.1 pounds. The difference in gain brought about by the alfalfa pressed juice (10.2 pounds) approaches significance.

TABLE 4.—GAIN OR LOSS IN WEIGHT OF EWES ON DIFFERENT ROUGHAGES DURING LACTATION

Lot No.	Ration[a]	No. of ewes started in lactation experiment	No. of ewes eliminated during observation period[b]	No. ewes observed through lactation	Average starting weight	Average weight at end of lactation[c]	Average gain or loss in weight
					pounds	*pounds*	*pounds*
1	Dehydrated alfalfa	10	1	9	114.3	111.7	− 2.6
2	Dehydrated alfalfa	10	0	10	108.7	113.8	5.1
3	Dehydrated alfalfa	10	0	10	113.5	102.3	−11.2
4	Dehydrated alfalfa	10	0	10	115.5	108.1	− 7.4
5	Dehydrated oat grass	10	0	10	118.6	124.9	6.3
6	Oat straw	10	2	8	116.3	101.6	−14.7
7	Field cured alfalfa	10	2	8	111.0	113.8	2.8
8	Alfalfa press juice	9	1	8	113.8	126.5	12.7
9	Alfalfa press cake	10	0	10	113.5	108.6	− 4.9
10	Alfalfa fat solubles	10	1	9	118.3	114.5	− 3.8
11	Alfalfa oil cake	9	0	9	120.8	112.2	− 8.6
12	Recombined alfalfa	10	0	10	113.9	109.1	− 4.8
13	Cottonseed meal	9	0	9	117.9	113.7	− 4.2
14	Casein	10	0	10	117.3	112.9	− 4.4

[a] See table 1 for composition of rations.
[b] These ewes were lost to the experiment because of death or loss of their lambs.
[c] Lactation period observed was the first eight weeks.

These two rations had similar protein contents (.30 and .33 pounds daily).

The high vitamin concentration of the pressed juice suggests the possibility of the importance of these or associated vitamins in sheep nutrition. The relatively high potency of the pressed juice in ascorbic acid is of special interest. The alfalfa pressed cake, oil cake and fat solubles had somewhat less nutritive value (lots 9, 10 and 11) than the pressed juice.

Further data on the value of the different rations studied is presented in table 4 which covers the performance of the ewes during the first eight weeks of lactation. It was the experimental plan to select the 10 ewes lamb-

ing closest together for the lactation studies, in order to reduce variation that might be related to seasonal environment changes, etc. This was not possible in all cases and less than 10 ewes were observed in some lots. The starting and finishing weights for this period were calculated only for the ewes that were observed during an entire lactation period of 8 weeks.

All ewes included in the calculations suckled only one lamb. In cases where ewes had twins one of the lambs was sacrificed in order to permit more critical analysis of the performance of both ewes and lambs. The ewes in the majority of the lots lost some weight, during the lactation period,

TABLE 5.—REPRODUCTION RECORD AND WOOL CLIP OF EWES FED DIFFERENT RATIONS

Lot No.	Ration[a]	No. of ewes	No. of twin lambs	No. of single lambs	Total lambs born	Average birth weight of twin lambs	Average birth weight of single lambs	Average birth weight of all lambs	Average wool clip	Difference[b]
						pounds	*pounds*	*pounds*	*pounds*	*pounds*
8	Alfalfa press juice	11	8	7	15	7.73	9.73	8.48	6.12	.05
6	Oat straw	13	6	10	16	7.09	8.68	8.08	6.17	.10
13	Cottonseed meal	12	4	10	14	8.47	9.31	9.06	6.27	.34
3	Dehydrated alfalfa	13	14	6	20	7.54	8.74	7.89	6.61	.04
7	Field cured alfalfa	12	10	7	17	7.82	9.76	8.67	6.65	.17
10	Alfalfa fat solubles	13	6	10	16	8.41	9.99	9.56	6.82	.08
11	Alfalfa oil cake	12	8	8	16	8.14	8.99	8.57	6.90	.05
4	Dehydrated alfalfa	12	4	10	14	9.55	9.06	9.20	6.93	.03
12	Recombined alfalfa	13	4	11	15	7.33	9.68	9.00	6.95	.02
2	Dehydrated alfalfa	11	4	9	13	7.09	8.77	8.26	7.39	.44
9	Alfalfa press cake	12	4	10	14	8.83	9.46	9.29	7.41	.02
14	Casein	12	6	9	15	9.39	9.20	9.27	7.59	.18
1	Dehydrated alfalfa	13	10	8	18	7.39	7.92	7.60	7.63	.04
5	Dehydrated oat grass	13	14	6	20	7.72	9.36	8.29	8.55	.92
Total		172	102	121	223	—	—	—	—	—

[a] See table 1 for composition of rations.

[b] Least significant mean difference by analysis of variance = 0.97 pounds.

varying from 2.6 to 14.7 pounds per ewe. The greatest loss in weight was in lot 6 (oat straw) which also proved to be the poorest ration in other respects. In lots 2 (dehydrated alfalfa), 5 (dehydrated oat grass), 7 (field cured alfalfa) and 8 (alfalfa press juice) the ewes gained in weight during lactation. It is of interest that the ewes in lot 8 gained the most and were the heaviest at the end of the lactation period. The slight loss in weight of the ewes in lot 1 (dehydrated alfalfa) can not be explained by the data.

Table 5 shows the reproduction record and wool clip of the ewes. The total number of lambs born in each lot varied from 13 to 20 with no definite correlation with the ration fed. The number of twin lambs born in each lot

varied from 4 to 14. The largest number of twins was found in the lots receiving the better rations although this would not seem to have any theoretical basis and was probably due to chance. Death losses were not related apparently to the ration fed although the lot receiving the best ration (lot 5, dehydrated oat grass) had only one death loss and the poorest ration (lot 6, oat straw) had 4. Conversely there were four deaths in lot 1 (dehydrated alfalfa), six in lot 7 (field cured alfalfa) and four in lot 8 (alfalfa press juice).

TABLE 6.—GROWTH OF LAMBS FROM EWES FED DIFFERENT RATIONS. PERIOD OF OBSERVATION 8 WEEKS

Lot No.	Ration of ewes[a]	Total no. of lambs[b]	Average birth weight[c]	Average final weight 8 weeks	Adjusted mean gain in weight[d]	Difference in gain[e]
			pounds	*pounds*	*pounds*	*pounds*
6	Oat straw	8	8.42	25.17	19.41	—
13	Cottonseed meal	9	9.64	30.63	20.99	1.58
4	Dehydrated alfalfa	10	9.63	32.90	21.90	.91
10	Alfalfa fat solubles	9	9.88	33.55	23.55	1.65
12	Recombined alfalfa	10	9.22	34.18	24.96	1.41
14	Casein	10	9.83	35.12	25.30	.34
8	Alfalfa press juice	8	8.72	35.51	27.72	2.42
11	Alfalfa oil cake	9	8.77	36.55	27.78	.06
9	Alfalfa press cake	10	9.01	37.18	28.17	.39
7	Field cured alfalfa	8	9.55	41.05	29.45	1.28
3	Dehydrated alfalfa	10	8.34	37.38	31.66	2.21
2	Dehydrated alfalfa	10	8.38	37.83	31.94	.28
1	Dehydrated alfalfa	9	8.37	39.73	33.93	1.99
5	Dehydrated oat grass	10	8.48	41.75	35.35	1.42

[a] Lambs were allowed access to ewes ration. See table 1 for composition of rations.

[b] Only lambs born relatively close together were observed; for milk production data on mothers during the same period see table 7.

[c] In case of twins one lamb was sacrificed; only one lamb nursed ewe in all cases.

[d] Adjusted to the same average birth weight by analysis of covariance; least significant mean difference in gain =2.88 pounds.

[e] Each figure indicates difference between that and the preceding lot.

In all cases except one (lot 4) single lambs weighed considerably more at birth than twins. Inspection of the average birth weights of single lambs, twins, or the combined weights shows no significant relationship to the ration fed.

A highly significant multiple regression relation of wool clip on protein consumption and net energy was found. Deviations from multiple regression were not significant indicating that when adjusted to the same level of protein and energy intake no significant differences existed between the lots.

The growth of the lambs from the ewes on the various roughages and the

milk production of the ewes is given in tables 6 and 7. During the first several weeks lambs are dependent mainly on their mothers' milk for food and their growth will be determined largely by the quantity of milk available and its quality. The data in tables 6 and 7 show that growth varied directly with the quantity of milk produced by the ewe. The simple correlation between these two factors was found to be 0.89. It is possible that differences in milk quality existed between lots but because of the difficulty in carrying out such determinations studies of this type were not made. The lambs in the lots whose mothers received decreasing amounts of dehydrated alfalfa

TABLE 7.—MILK PRODUCTION OF EWES FED DIFFERENT ROUGHAGES

Lot No.	Ration[a]	No. of ewes	No. of observations	Mean day in lactation	Mean daily milk per ewe	Adjusted mean daily milk per ewe[b]	Differences in milk production[c]
					ounces	*ounces*	*ounces*
6	Oat straw	8	64	29.0	12.3	12.2	—
13	Cottonseed meal	9	72	30.7	15.6	15.6	3.4
4	Dehydrated alfalfa	10	80	29.7	16.4	16.3	.7
12	Recombined alfalfa	10	80	28.6	16.8	16.6	.3
10	Alfalfa fat solubles	9	72	29.4	17.7	17.8	1.2
3	Dehydrated alfalfa	10	80	28.5	18.3	18.1	.3
2	Dehydrated alfalfa	10	80	29.9	18.8	18.8	.7
11	Alfalfa oil cake	9	72	30.6	19.3	19.3	.5
14	Casein	10	80	31.6	19.3	19.4	.1
8	Alfalfa press juice	8	64	32.3	19.4	19.6	.2
9	Alfalfa press cake	10	80	31.1	20.9	21.0	1.4
7	Field cured alfalfa	8	64	30.1	21.8	21.8	.8
1	Dehydrated alfalfa	9	72	30.1	21.8	21.8	.0
5	Dehydrated oat grass	10	80	29.2	25.0	24.9	3.1

[a] See table 1 for composition of rations.

[b] Adjusted to general mean day of lactation (30.03 days) by analysis of covariance. Least significant mean difference = 1.98 ounces.

[c] Each figure indicates difference between that and the preceding lot.

(lots 1, 2, 3, 4) grew at decreasing rates respectively. However, the lot receiving the lowest allowance of dehydrated alfalfa (lot 4) grew at a faster rate than the lot receiving oat straw (lot 6). Lot 6 lambs grew at the slowest rate of any and were generally unthrifty. The lambs of the ewes fed oat grass (lot 5), made the most rapid gains and were the most uniform in weight and finish. All of the alfalfa fraction lots (8 to 12 inclusive) grew at reduced rates as compared either to dehydrated alfalfa, field cured alfalfa or oat grass and did not show the thrift and vigor of lambs in these lots. The same may be said for the lots receiving the protein supplements (Nos. 13 and 14).

The final market grades of the lambs at approximately 100 days of age are of interest. As previously discussed there was a correlation of 0.89 between milk production of the ewes and weights of the lambs at 8 weeks; and by inspection of the data it appears there is a general relationship between lamb weight at 8 weeks and final market grade. The greatest number of choice lambs appeared in lots 5, 1, and 2, the lots in which the most satisfactory gains were made. It would seem, therefore, that the growth and thrift of the lamb during its first 8 weeks is of considerable importance in determining its final market value.

The data on the growth of the lambs was treated by the analysis of covariance. Highly significant differences in gain were found between lots 6 (oat straw) and all of the other lots except 13 (cottonseeed meal) and 4 (low level of dehydrated alfalfa). It is again of interest to compare the gains of the lambs in lots 13 and 8 (cottonseed and pressed juice of alfalfa). The difference in gain was 6.73 pounds and is statistically significant.

As previously mentioned above milk production of the ewes (table 7) correlated to a high degree (0.89) with the gains of the lambs at 8 weeks. Milk production was greatest in the lambs from lot 5 (oat grass), followed in decreasing order by lots 1 and 7 (dehydrated and field cured alfalfa). It was the least in lot 6 (oat straw). The other lots were intermediate in milk production. It is interesting to compare the wide difference in milk production of the ewes fed the dehydrated oat grass (25 ounces of milk per ewe, per day) and those receiving the oat straw (12.3 ounces). These data demonstrate a rapid deterioration in the nutritive value of the oat plant harvested at different stages of growth.

Discussion

The data obtained in this experiment indicate the high nutritive value contained in both dehydrated and field cured alfalfa and the very superior value of early cut dehydrated oat grass for the maintenance of breeding ewes and the growth of their lambs. The experiments do not show definitely whether these nutritive factors are in the nature of protein, known vitamins, substances at present unknown, or combinations of these factors. The fact that casein, a protein of high biological value, did not entirely supplement the oat straw ration, and the results obtained with the pressed juice of alfalfa would suggest the conclusion that protein alone is not the limiting factor in these experiments.

The data were treated by an analysis of multiple regression in an effort to evaluate the effect of the amount of protein and energy in the rations. As already mentioned most of the variation in wool clip between lots was found to be attributable statistically to the amount of protein and energy

in the rations ($R^2 = .75$). In the case of gains of ewes during pregnancy 58 per cent of the variation between lots was due to multiple regression of gain of protein and energy, with gains of lambs 66 per cent, and with milk production of ewes 76 per cent. However, except in the wool clip data, significant deviations from multiple regression exist indicating that other factors than protein and energy content were operative.

Vitamin A would likewise appear to be eliminated as a factor as demonstrated in earlier work (Shrewsbury, *et al.*, 1942). It appears that the water soluble fraction of the alfalfa, and probably of other plants, contains a factor or factors necessary for the best nutrition of sheep. Research to identify this factor or factors with known vitamins or other substances is at present in progress.

Conclusions

1. Dehydrated oat grass was found to have a high nutritive value as a roughage for breeding ewes as judged by growth, maintenance, and milk production and for the production of thrifty, rapidly growing lambs.

2. It was found that 2.5 pounds of dehydrated alfalfa were required for good growth, maintenance, and milk production of breeding ewes when the basal ration was composed of yellow corn and corn silage.

3. Casein or cottonseed meal did not completely supplement the basal oat straw, corn, corn-silage ration for breeding ewes.

4. The water soluble fraction of alfalfa (pressed juice) was found to have definite nutritive value for breeding ewes.

5. The deficiencies of the basal ration of oat straw, corn and corn silage were found to be protein, vitamin A and factors contained in the water soluble fraction of alfalfa.

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VARIATIONS IN THE OCCURRENCE OF BLOAT IN THE STEER PROGENY OF BEEF BULLS

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BLOAT in beef cattle is considered by many feeders as one of the principal causes of loss in feed-lot operations. This condition has been investigated for many years but as yet not all the physiological causes in the production of bloat are understood.

McIntosh (1938) divides bloat into two general classes: one in which the gas exists in a free state and collects in the upper part of the rumen, and the other in which the rumen is filled with a mass of fermenting material and the gas for the most part is intermingled with the food. McIntosh points out that the first type is probably due to an esophageal obstruction and regurgitation of gases cannot take place owing to this obstruction, and that the second type is encountered when cattle engorge with succulent food.

The pasturing of clover and alfalfa has been shown to be a source of bloat in both cattle and sheep (Cole, Mead, and Kleiber, 1942), although Schalk (1935) concluded that excessive bloat was not caused by condition of fill or dew conditions on pastures of this type. Cole, *et al.* (1942) advance the theory that expulsion of gas from the rumen by belching is a reflex mechanism dependent upon an adequate amount of fibrous material of a prickly nature. They point out that succulent legumes and concentrates contain a minimum of fiber and are therefore particularly conducive to bloat, and they suggest introduction of sufficient fiber in the ration to initiate belching, as a preventive measure.

Kephart (1929)and Cole, *et al.* (1942) state that bloat is to some extent an individual matter and occurs much more frequently in some animals than in others. Cole, *et al.* (1942) suggest that the chronic bloater may have a high threshold of nervous irritability, and that, in such cases, even coarse roughage is insufficient to stimulate belching.

The purpose of this paper is to present a study of observed differences between sire groups in the frequency of bloat. Practical feeders have often observed that the progeny of a particular bull take to heavy feeding of grain without any apparent digestive trouble while progeny of other bulls tend

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to be off feed or experience difficulty in handling large quantities of concentrates. It is this attribute that we are attempting to measure.

Material and Methods

The 13 bulls used to sire the calves fed out in connection with record of performance tests at the U. S. Range Livestock Experiment Station, Miles City, Montana, in 1940–41 and 1941–42 were all registered Herefords. The cows were in most cases high grade Herefords carrying better than 90 per cent of Hereford blood while the rest of the cows were registered Hereford cows. The T or "test" herds contained mostly grade cows and the L or line herds contained only purebred cows. There are two repetitions of sires for the two years, Bull T1 in 1940–41 was Bull L2 in 1941–42 and Bull T3 in 1940–41 was Bull L3 in 1941–42.

All steers were full fed a grain mixture of 6 parts of corn, 3 parts of dried molasses beet pulp, 1 part of wheat bran, and 1 part of linseed meal and a good quality alfalfa hay. In 1940–41 the feeding period was 265 days and in 1941–42 it was 252 days. Wherever possible, 8 steers sired by each bull were fed. The test herds were made up of cows divided at random and if an excess of 8 steer calves were produced the calves to be fed were chosen at random. The steers were fed the grain mixture twice a day in individual stalls. The feeder closely watched the steers during the feeding time and for about an hour after feeding. Bloating was determined by observing a swelling of the left side of the steer. In severe cases, a rubber stomach tube was used to relieve the gas pressure. Notations on barn feed record sheets were made of each occurrence of bloat. If a steer was noted as having bloated during any part of a day, it was recorded as one steer day with bloat.

The X^2 test was used to determine the significance between frequencies of occurrence of bloat (Fisher, 1941).

Data and Discussion

The incidence of bloat in the progeny of each of the bulls studied in the two years 1940–42 is shown in table 1. The frequency of bloat was much less in 1941–42 than in 1940–41, the average percentage of steer days with bloat being 3 per cent, and 1.7 per cent respectively. The highest frequency of bloat in 1940–41 was observed in the progeny of the T3 bull and the lowest was in the T2 bull. In 1941–42 the highest per cent of bloat was observed in the L2 bull and the lowest in the T5 bull. In this latter case only 1 of the 8 steers bloated 1 day during the 252 days of feeding.

The bull L3 in 1941–42 is the same bull as T3 in 1940–41. In both years his offspring had a fairly high frequency of bloat even though he was bred to 2 different groups of cows. Bull T1 in 1940–41 is the same bull as L2 in

1941–42. In the latter year the progeny of this bull had a higher percentage of bloat than during the preceding year. He was bred, however, to the same group of cows as the L2 in 1940–41.

A study of the distribution of the occurrence of bloat in table 1 indicates that only about 42 per cent of the steers fed exhibited no bloat at all and approximately 11 per cent could be considered excessive bloaters. Of those steers showing excessive bloat, approximately half of them were sired by one bull in the 2 years that he was used and another third of them traced to

TABLE 1.—INCIDENCE OF BLOAT IN THE PROGENY OF EACH BULL STUDIED

Year	Sire	Distribution of occurrence of bloat by steers								Steer days of bloat
		Number of days of bloat								
		0	1–5	6–10	11–15	16–20	21–25	26–50	51 or more	
										per cent
1940–41	T1	2	5	1	—	—	—	—	—	0.75
"	T2	4	4	—	—	—	—	—	—	0.38
"	T3	1	3	—	1	—	1	1	1	8.68
"	L1C	5	1	—	1	—	—	—	—	0.70
"	L2	2	2	1	—	1	—	1	—	4.37
1941–42	L2	1	6	—	—	—	—	—	1	4.38
"	L3	4	2	—	—	1	—	1	—	2.63
"	T1	4	2	2	—	—	—	—	—	0.84
"	T2	3	3	1	—	—	1	—	—	1.84
"	T3	1	6	—	—	—	—	1	—	2.08
"	T4	2	5	—	—	—	—	—	1	3.92
"	T5	7	1	—	—	—	—	—	—	0.05
"	T6	3	3	—	—	—	—	—	—	0.26
"	T7	5	3	—	—	—	—	—	—	0.40
"	T8	5	3	—	—	—	—	—	—	0.20

a common grandsire. Bloat was not found, however, in all steers from any of the sires, though in 3 cases there was only one steer out of each lot that did not bloat.

Inspection of the data in table 1 reveals considerable variation in the incidence of bloat between the progeny of different bulls. Data of this discontinuous type are rather difficult to handle in a statistical analysis and for this reason several approaches have been made, in any effort to determine if the observed differences between progeny groups were statistically significant.

An analysis of the data on steers exhibiting any bloat at all as compared with those that exhibited no bloat indicated that differences between prog-

eny groups were not significant in either year, using the X^2 test. If it is assumed, however, that steers exhibiting in excess of 10 days of bloat are chronic or excessive bloaters, and the X^2 test is applied for frequency of steers exhibiting excessive bloat, a significant difference was found between progeny groups in frequency of bloat in 1940–41, but not in 1941–42.

Another analysis was made, based on the number of steer days of bloat, rather than number of steers exhibiting bloat. Each of the lots of steers sired by different bulls was tested for homogeneity by the X^2 test. It was found that one group (T_2) in 1940–41 and 3 groups (T_5, T6, T8) in 1941–42 were homogeneous. In all the other sire groups, the significantly large values of X^2 point to greater variations between steers, within each sire group, than can be accounted for by chances of random sampling from homogeneous populations. A further X^2 test showed that a highly significant difference existed between progeny groups in the number of steer days of bloat in both years. These tests demonstrate two points; namely, that there are significant variations within the progeny of sires, and that there are significant variations between progeny groups by different sires. Wherever there are differences between sire groups, it is to be expected that there are differences also between the progeny by each sire. Even though most of the sire groups were found to be heterogeneous, this does not appear to invalidate the finding that greater variations were observed between sire groups than would be expected by chance. In other words the averages of the steers by various bulls differ enough from each other to make it clear that there were real differences between bulls in transmitting ability. These are differences over and above whatever chance differences between groups would have occurred due to the fact that not all of the progeny by each bull were exactly alike in frequency of bloat.

It has been shown above that in one year there was a statistically significant difference in the number of steers in progeny groups showing excessive bloat. The results also indicate that there was a significant difference in the frequency of bloat between the progeny of different sires in both years. In connection with these findings it is interesting to note that fewer digestive disturbances were observed (Phillips, *et al.*, 1942) in crossbred than in purebred steers while on feed.

A plausible explanation of the physiological basis for these differences between animals and sire groups of animals in frequency of bloat is difficult. All who have fed steers know that some steers can and will consume large quantities of concentrates without digestive disorders occurring. In lot-fed groups, some steers have been observed to spend a large part of the time eating grain and very little time eating hay or roughage. The steers used in this study were individually fed and did not have this opportunity to select

the type of feed they desired or could best handle. This probably results in more digestive disorders than with lot feeding but individual feeding does bring out differences in this respect. The steers that would naturally consume more concentrates and less roughage if they had the opportunity to do so are probably little affected by individual feeding but those that desire more roughage are forced to eat more concentrates than they can handle and thus experience digestive disorders. Significantly more digestive disorders were observed with individually-fed steers when compared with group-fed steers in an experiment conducted by the Bureau of Animal Industry and the Texas Agricultural Experiment Station.[2] It may be that this apparent variation in occurrence of bloat is due to a difference in the level of nervous reaction or threshold of stimulation in the rumen, as suggested by Cole, *et al.* (1942). This subject warrants further study since it is of considerable practical importance to the cattle producer and feeder.

Summary and Conclusions

A study was made of the frequency of bloat in steer calves on full feed sired by 13 purebred Hereford bulls used in 2 successive years at the U. S. Range Livestock Experiment Station, Miles City, Montana. A significant difference in number of steers showing excessive bloat was observed between the progeny of various bulls in one year. An analysis based on the number of steer days showed that a highly significant difference existed between progeny groups in both years in the frequency of bloat. These results indicate that there are inherent differences between the progeny of different bulls in ability to handle large quantities of feed without digestive disturbances, and indicate the possibility of improvement in this characteristic by selection on the basis of progeny tests.

[2] Unpublished data.

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DETERMINATIONS OF METABOLIZABLE ENERGY OF FEEDING STUFFS FOR CATTLE[1]

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WHILE the measurement of total nutritive values of individual feeding stuffs seems to be an impossible objective, from a critical point of view, the need for such measures, to serve as guides in feeding practice, requires continuing search for the most practicable compromise with scientific conceptions of the problem.

In this spirit—after the determination of the digestible nutrients of feeding stuffs, the next step toward more significant measurements of nutritive value is the determination of metabolizable energy, that is, the gross energy minus the energy of the feces, the urine (corrected for the nonmetabolizable energy of the protein stored) and the methane.

What is the most practicable method of accomplishing this purpose?

In an effort to guide the way toward this objective the authors present first, as bases of comparison, a series of experimental measurements of the percentage of the gross energy of a normal, dry ration of alfalfa hay, corn meal and linseed meal which is metabolizable, and three series of similar computed values of this ration, and second a series of experimental measurements of the percentage metabolizability of a group of silages, and four parallel series of computed values of the same—these values for the silages being determined by difference between results for the dry ration first mentioned and for the same plus the silages.

The first column in table 1 presents the experimental measurements of the percentage metabolizability of the basal ration and of the silages.

In the second column are parallel values derived from experin ental measurements of feces and urine, and values for methane computed by means of the formula of Bratzler and Forbes (1940), which relates the methane to the digestible carbohydrates of the feeding stuffs.

In the third column are similar values for metabolizable energy computed from experimental measurements of feces and urine and values for methane computed by means of the formula of Kriss (1930), which relates the methane to the dry matter of the ration.

These two sets of computed values agree remarkably well with the experimentally determined values. The average difference between the observed and the computed values for methane involved in the derivation of

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these formulae was 8.4% for Bratzler's formula and 10.6% for the formula of Kriss.

These two computations of metabolizable energy from experimentally determined values for feces and urine, and computed values for methane, however, have against their practical usefulness the fact that the energy

TABLE 1.—COMPARISON OF VALUES FOR THE PERCENTAGE OF THE GROSS ENERGY OF A BASAL RATION AND OF SILAGES WHICH IS METABOLIZABLE

Steer No.	Character of rations	Experimentally determined[1]	Computed; Bratzler[2]	Computed; Kriss[3]	Computed; Axelsson[4]	Computed; Axelsson[5]
14	Alfalfa hay, corn meal, linseed oil meal	57.4	57.8	58.0	59.8	
14	Alfalfa hay, corn meal, linseed oil meal	58.4	58.8	59.1	61.1	
16	Alfalfa hay, corn meal, linseed oil meal	59.7	59.4	59.8	61.1	
16	Alfalfa hay, corn meal, linseed oil meal	60.2	59.7	60.1	62.0	
24	Alfalfa hay, corn meal, linseed oil meal	60.1	60.8	61.2	63.8	
25	Alfalfa hay, corn meal, linseed oil meal	56.1	55.5	55.5	57.0	
26	Alfalfa hay, corn meal, linseed oil meal	59.6	59.7	60.0	61.5	
14	Basal+alfalfa-molasses silage	50.8	52.3	49.7	56.7	51.7
16	Basal+alfalfa-molasses silage	52.2	55.4	53.6	58.4	53.3
14	Basal+alfalfa-phosphoric acid silage	48.1	49.9	46.9	59.9	48.3
16	Basal+alfalfa-phosphoric acid silage	43.7	47.8	45.5	52.4	47.2
14	Basal+alfalfa-phosphoric acid silage+limestone	51.0	51.9	49.9	53.4	49.3
16	Basal+alfalfa-phosphoric acid silage+limestone	47.9	50.3	47.7	52.5	48.3
24	Basal+clover-timothy-molasses silage	43.6	43.5	41.2	50.0	42.1
24	Basal+soy-bean-molasses silage	48.5	50.0	47.5	54.8	47.6
25	Basal+corn silage	52.7	53.8	52.9	64.4	49.7
26	Basal+soybean-sorghum silage	39.3	41.4	39.2	48.2	40.0

[1] Determined from observed values for feces and urine, and gravimetric measurement of methane.
[2] Computed from observed values for feces and urine, and Bratzler and Forbes's formula for methane.
[3] Computed from observed values for feces and urine, and Kriss's formula for methane.
[4] Computed from Axelsson's factors, including 7.8 Cal. per gm. for ether extract of roughages.
[5] Computed from Axelsson's factors except for the use of 3.3 Cal. per gm. for either extract of silages.

values for the feces and urine of feeding stuffs in general are not now available.

We pass, therefore, to the fourth column of data in table 1, which sets forth metabolizable energy values computed from the factors of Axelsson (1941), which are derived from 71 energy balance experiments with cattle conducted by Kellner.

These factors represent the metabolizable energy per gram of digestible crude protein, ether extract, nitrogen-free extract and crude fiber.

The values thus computed agree well with the experimentally determined values of the dry, basal ration, but not well with the similar values for the several silages fed superimposed upon this basal ration.

One apparent error involved in the computation of the metabolizable energy of the silages from Axelsson's factors seems to be that the factor of 7.8 calories per gram for the metabolizable energy of the ether extract of roughages is too high to represent the ether extract, especially the low-carbon ether-soluble organic acids, of the silages. When a factor (which the authors supply) of 3.3 calories instead of 7.8 calories per gram of digestible

TABLE 2.—FACTORS FOR CALCULATION OF METABOLIZABLE ENERGY OF FEEDING STUFFS FOR CATTLE FROM THEIR CONTENTS OF DIGESTIBLE NUTRIENTS

Nutrients		Metabolizable energy per gm. digestible organic matter
		kcal.
Protein:	roughage	4.30
	concentrates	4.50
Ether extract:	roughage	7.80
	grains	8.30
	seeds of oil plants	8.80
	foods of animal origin	9.30
Carbohydrates:	polysaccharides	3.76
	trisaccharides	3.62
	disaccharides	3.56
	monosaccharides	3.38
	nitrogen-free extract (average)	3.70
	crude fiber	2.90

ether extract is used with Axelsson's other factors, his method of calculation, as shown by the fifth column of data, is satisfactory for silages. With this modification, therefore, Axelsson's system of calculation of metabolizable energy of feeding stuffs seems to be the most practicable that is now before us.

In the application of Axelsson's factors to the basal ration, as in table 1, it is necessary to state that inasmuch as Axelsson proposes different factors for the digestible protein and ether extract of roughages and concentrates; as this basal ration was composed of roughage and concentrates in equal quantities; and as there was no way of separating the digestible nutrients of the individual feeds of which the ration was composed, the authors used averages of Axelsson's factors for the metabolizable energy of the protein

and of the ether extract of roughages and of concentrates in computing the metabolizable energy of this ration.

It is also necessary to explain that the factor of 3.3 which is used for computing the metabolizable energy of the digestible ether extract of the silages was derived by first computing the metabolizable energy of the protein and the carbohydrate of the silages, by the use of Axelsson's factors for these constituents of roughages, and then assigning to the metabolizable energy of the ether extract of the silages the difference between the sum of the quotas computed for protein and carbohydrates and the metabolizable energy of the silage as a whole, as determined by experiment. This factor of 3.3, which was used for computing the metabolizable energy of the digestible ether extract of silages, therefore, is a highly conventional value which covers all errors in Axelsson's entire system of computation.

In further discussion of the validity of the computed values presented—there appears to be an element of error in the formula of Bratzler and Forbes, which considers the methane to be derived from digestible carbohydrate alone, this error depending on the same fundamental fact that is responsible for the error already pointed out in Axelsson's factor for metabolizable energy of the ether extract of roughages when applied to silages.

Thus, the chemical analysis of silages, by the usual proximate procedure, assigns to ether extract certain low-carbon organic acids which are not fats, and which presumably contribute to the formation of methane. The values for digestible carbohydrate of silages, therefore, are presumably too low accurately to represent the methane-forming fraction of these feeding stuffs.

These related errors, in the formula of Bratzler and Forbes, when used to compute the methane formed from silage, and the factor of Axelsson for metabolizable energy of the digestible ether extract of roughages, as this value is applied to silages, both result in improperly high values for the metabolizable energy of silages, the error due to the formula of Bratzler and Forbes being relatively slight, since it affects a relatively large multiplicand, while the error in the stated use of Axelsson's factor is much larger because it affects a relatively small multiplier.

In comparing the relative validity of the formula of Bratzler and Forbes and the formula of Kriss, for computing methane production, as affecting values for metabolizable energy—the formula of Bratzler and Forbes gives slightly more accurate results in relation to feeding stuffs other than silages, while Kriss's formula gives more accurate results for silages—for the reason that the dry matter of silages includes all of the nutrients from which methane is derived, while the digestible carbohydrate of silages, as ordinarily determined, seems not to do so.

For convenience of reference the following table of factors for the com-

putation of metabolizable energy from digestible nutrients is quoted from the work of Axelsson:

The method of Axelsson, therefore, for computing the metabolizable energy of feeding stuffs for cattle, by multiplying the values for the digestible nutrient contents of the feeds by appropriate factors, seems to afford the most practicable procedure thus far proposed for computing these measures.

The factor of 3.3, which is proposed by the authors of this paper for use in computing the metabolizable energy of the digestible ether extract of silages, is that value which when used with Axelsson's factors for digestible protein and carbohydrate of roughages, yields total metabolizable energy values of silages in approximate agreement with values based entirely on experimental observations.

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A STUDY OF THE CHARACTERISTICS OF LIVE MARKET HOGS AS RELATED TO THE QUALITY OF CARCASSES PRODUCED

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Introduction

THE great amount of variation in the depth of back fat and the size of the eye of lean in the loin of hog carcasses from pigs of similar weights of the same and of different breeds led to the studies reported in this paper. The fatness of the live hog is one of the major characteristics which determine the value of market hogs. On the other hand, the average consumer prefers retail cuts that contain a moderate amount of fat and a high proportion of lean meat. It would be helpful to be able to determine by inspection the degree of fatness of the live hog that is desired in order to satisfy the consumer of retail cuts. It would be desirable also to determine the relationships that may exist between such characteristics as thickness of back fat, live weight, dressed weight, and amount of lean meat in certain retail cuts.

Extensive studies of carcasses yielded by various types of swine have been made by Bull and Longwell (1929), Bull, *et al.* (1935) and Hankins (1940). These studies show that as the hogs become fatter the proportion of the lean cuts of the carcass decreases and the proportion of the fat cuts increases. Warner, Ellis and Howe (1934) showed that there was a consistent relationship between the content of fat in the edible portion of the hog carcass and the percentages that the weights of certain cuts bear to the carcass weight. Hankins and Ellis (1934) have demonstrated that the thickness of back fat may be regarded as a dependable, practical indication of the fatness of a hog carcass. McMeekan (1941) found that there was a high correlation between various measurements of the thickness of the backfat and the total weight of the fat in the carcass. The results of studies of bacon carcasses by Winkler, White and Abel (1941) indicate that good quality in a bacon carcass varies directly with the size of the eye of lean, and indirectly with the fat content.

Experimental Animals

The animals used in these studies were spring and fall farrowed Berkshires, Chester Whites, Duroc Jerseys and some crosses of these breeds. All

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pigs were bred and fed in the station herd. The pigs had been full-fed from weaning to slaughter dates. A large percentage of the pigs had been used in feeding experiments from a short time after weaning until a final average weight per lot of 180 or 200 pounds had been reached. They were slaughtered in the station meat laboratory usually in groups of four or five head. Corn was the chief grain fed and the differences in the rations offered were usually confined to the kinds of protein and vitamin supplements. It is believed that the rations did not influence appreciably the quality of the carcasses produced. These pigs were of the intermediate type and were selected for slaughter on the basis of the finish they carried.

Experimental Procedure

As a rule the final live weight was obtained after the pigs had been off of feed for a period of from three to five hours. Warm dressed weights were used in determining dressing percentages. The carcasses were split through the center of the backbone. The carcass weight included the head and leaf fat and the hams were not faced.

The pigs were graded on foot by one or more persons according to their condition or degree of fatness and certain measurements were made of the thickness of back fat on the carcasses. The latter measurements were obtained at the first and the last thoracic and the last lumbar vertebra. In these studies the average of the three measurements was used. The thickness of back fat was measured exclusive of the skin.

Certain measurements were made of the cross-section of the loin-eye muscles at the junction of the thoracic and lumbar regions. During 1939–40 and 1940–41 an effort was made to estimate the area of the cross-section of this muscle by multiplying its length by its width. This method was discarded in 1941–42 because a more accurate method was used. The latter method involved the use of a compensating polar planímeter upon a tracing of the cross section of the eye of the loin.

The ham was removed behind the second sacral vertebra by cutting at a right angle to the leg. The area of the lean portion of the ham at this point was determined by the use of a planimeter upon tracings of this cut. Also, during 1941–42, a record was made of the circumference of the ham at a right angle to the axis of the leg at the uppermost point of the aitchbone as the carcasses hung in the cooler.

In the preliminary trial conducted during 1939–40 a record was made of the apparent firmness of the live hogs just before slaughter. Firmness was estimated by handling the hogs over the back, loin and rump in a manner similar to that used in judging sheep or cattle. This was done to learn whether or not it is possible to estimate the thickness of back fat on the live hog.

Results and Discussion

The studies conducted in 1939–40 and 1940–41 are considered as preliminary and are discussed briefly. The work done in 1941–42 is reported as a separate experiment.

Preliminary Studies

A total of 268 market pigs was used in the first two years of work. Records obtained from 214 of these show a highly significant correlation of +0.42 between the condition (fatness) as determined by the visual observation of the live hog and the thickness of back fat.

A highly significant correlation coefficient of +0.47 was obtained between liveweight and back fat. A study involving 217 head (77 Berkshires, 34 Chester Whites, 63 Duroc Jerseys and 43 crossbreds) showed no correlation between firmness (as determined by handling) and thickness of backfat. This lack of correlation was noted even when the breeds were considered separately and when certain groups with a small range in weight were studied.

The 1941–1942 Results

A total of 125 pigs from the spring and the fall pig crops was used in this work. There were 30 Chester Whites, 40 Duroc Jerseys, 48 Berkshires and 7 crossbreds in this study. Thirty head each of the Berkshires and the Duroc Jerseys selected at random and all of the Chester Whites were used for detailed study. This selection was made to facilitate the handling of the data statistically. The correlation coefficients for six characteristics of 90 live pigs and of their carcasses are given in table 1. These values are based on the variation within breeds as derived by an analysis of covariance.

The correlation between liveweight and the circumference of the ham was also highly significant. The correlation coefficient was +0.86. If this value is squared the coefficient of determination is obtained which indicates that approximately 78 per cent of the variation in the circumference of the ham was associated with corresponding changes in the live weight.

Somewhat lower but still highly significant correlations were obtained between live weight and thickness of back fat, live weight and the area of the end cut of the ham, and live weight and the area of the loin eye muscle. These values were +0.47 for the back fat, +0.60 for the end cut of the ham, and +0.28 for the area of the loin eye. It is interesting to note that only about 8 per cent of the variation in the area of the loin eye was associated with differences in live weight; whereas about 36 per cent of the variation in the area of the end cut of the ham was associated with changes in live weight.

It may be noticed in table 1 that the dressed weight (warm) is more highly

correlated with the thickness of back fat and with the area of the loin eye muscle than is the live weight with these same characteristics. The dressed weight is highly correlated with the circumference of ham and the area of the end cut of the ham.

There was no significant correlation between the thickness of back fat and the area of the loin eye muscle or the area of the lean of the end cut of the ham. However, there was a highly significant correlation between the

TABLE 1.—SIMPLE CORRELATION COEFFICIENTS* ON SIX CHARACTERISTICS IN LIVE AND DRESSED 1941 SPRING AND FALL PIGS—30 HEAD EACH OF THE BERKSHIRE, CHESTER WHITE AND DUROC JERSEY BREEDS

	Dressed weight (warm)	Black fat average thickness	Area of loin-eye muscle	Circum. ham	Area of lean in end cut of ham
	pounds	*cm.*	*sq. in.*	*inches*	*sq. in.*
Live weight pounds	+.96	+.47	+.28	+.88	+.60
Dressed weight (warm) pounds		+.52	+.35	+.88	+.60
Back fat av. thickness cm.			−.11	+.44	+.01
Area of loin-eye muscle sq. in.				+.42	+.53
Circum. ham, inches					+.58

* All of the correlation coefficients above are considered highly significant except those between thickness of back fat and area of loin-eye muscle, and thickness of back fat and area of end cut of the ham.

thickness of back fat and the circumference of the ham. The circumference of the ham is influenced by the fatness of the pig.

The coefficient of correlation between the area of the loin eye and the circumference of the ham was +0.42 and between the area of the loin eye and the area of the lean of the end cut of the ham was +0.53. The coefficients of determination were 17 and 28, respectively. McMeekan (1941) has previously pointed out that suitable combinations of internal linear measurements of muscle obtained on the cross section at the junction of the thorax and loin are highly correlated with the weight of muscle in the carcass and are capable of providing a reliable basis for its estimation.

A coefficient of +0.58 was obtained when the circumference of the ham and the area of the lean of the end cut of the ham were correlated. Only 33 per cent of the variation in the area of the end cut of the ham was associated with variations in the circumference of the ham. Hankins (1935) showed a

high correlation between plumpness and hams and the ratio of meat to bone and also that fatness played an important part in plumpness.

A study of the mean values shown in table 2 reveals some differences in the breeds of the pigs studied. It should be borne in mind, however, that the values in this table were obtained from a small number of pigs in one herd under one system of management and may or may not be representative of these breeds throughout the country. An analysis of variance showed that the Chester Whites and Duroc Jerseys used in this work carried significantly greater depths of back fat than the Berkshires. There were no sig-

TABLE 2.—COMPARATIVE MEAN VALUES OF SEVERAL OF THE CHARACTERISTICS STUDIED

Breed of swine	No. of animals	Age in days	Live weight when slaughtered	Weight of warm carcass	Thickness of back fat	Area of loin eye muscle	Circumference of ham	Area of lean in end cut of ham
			lbs.	*lbs.*	*cm.*	*sq. in.*	*inches*	*sq. in.*
Berkshire	30	222.5	206.8	168.6	3.68	5.71	24.49	16.09
Chester White	30	211.4	201.8	164.3	4.25	5.09	24.89	14.79
Duroc Jersey	30	213.9	217.4	173.8	4.15	4.49	24.82	14.30
All breeds	90	215.9	208.7	168.9	4.02	5.10	24.73	15.06

nificant differences in the size of the muscle of the end cut of the ham in the Chester Whites and Duroc Jerseys. The Chester Whites used in this study had a larger loin eye than the Duroc Jerseys, but a smaller loin eye than the Berkshires. There was considerable variation within breeds in the several characteristics studied.

Summary

These studies involved the use of records from 393 spring and fall farrowed pigs produced in the Cornell University Experiment Station herd. The Berkshire, Chester White and Duroc Jersey breeds and their crossbreds were included in the studies. The pigs were full-fed from weaning time.

An analysis of the data collected during 1939–40 and 1940–41 shows a highly significant correlation between condition as determined by visual observation and the average thickness of back fat in the carcasses. On the other hand it was not possible to estimate the thickness of back fat by handling the live hogs before slaughter. The thickness of the back fat was greater as the live weight of the hogs increased and, as expected, there was a highly significant correlation between live and dressed weights.

The results of the work done in 1941–1942 are in accord with that of the previous work in the relationship between live weight and thickness of back fat and also between live weight and dressed weight.

The results of detailed studies of six characteristics involving 30 head each of the Berkshire, Chester White and Duroc Jersey hogs made to determine certain other relationships are shown in table 1.

Highly significant correlations were obtained between several of the factors studied, however, there was little or no correlation between the fatness of the pig and the development of loin-eye muscle or the lean in the end cut of the ham.

The area of the loin-eye muscle is correlated with weight but less so than with several other factors studied.

There was a highly significant correlation between the area of the loin-eye muscle and the area of the lean in the end cut of the ham.

The circumference of the ham is influenced by the fatness of the pig.

Certain differences were found between the breeds studied, but more extensive work should be done with hogs from different sources before general conclusions are drawn.

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CHEMICAL COMPOSITION, GRAZING VALUE AND VEGETATIVE CHANGES OF HERBAGE IN A TYPICAL PLAINS PASTURE

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IN SPITE of the drought and dust storms of recent years, several million acres of grass land are utilized for livestock production in the Southern High Plains, which includes southwestern Kansas, southeastern Colorado, the Oklahoma Panhandle, northwestern Texas and northeastern New Mexico. In the three Panhandle counties of Oklahoma alone there are approximately 1,750,000 acres of native grass land, which represent 49% of the total acreage of this area.

The predominating grasses in this general area are buffalo grass (Buchloe dactyloides) and blue grama (Bouteloua gracilis), which are found especially on the heavier upland souls, as pointed out by Hoover (1939) and Savage (1939). Russian thistles (Salsola pestifer) have become wide-spread, particularly on pastures that have been damaged by wind erosion and drought. During the unusually dry years, from 1934–39, a large part of the grazing from the small ranch and farm pastures was furnished by Russian thistles. Their excellent feed value for pasture and hay is described by Cave, Riddell and Hughes (1936); Christensen (1934); and Morton, *et al.* (1932); and others.

Although Savage and his co-workers (1937, 1939, 1940) have reported work on drought survival, regrassing recommendations and range improvement practices, there has been little information published concerning the chemical composition, grazing value and vegetative changes of the principal grasses of the Southern High Plains area.

Several observers have reported chemical analyses of blue grama, including Hopper and Nesbitt (1930), Stanley and Hodgson (1938) and Watkins (1937). None of these studies, however, have been conducted under Southern Great Plains conditions.

The purpose of this paper is to discuss the results of studies begun in May, 1939 regarding (1) the chemical composition, (2) grazing value and (3) vegetative changes of herbage in a typical upland plains pasture.

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Materials and Methods

An enclosure about 20×64 feet was established in the spring of 1939 in a part of a 32-acre-terraced-experimental pasture where blue grama was the predominating species. Four meter-square quadrats with pure stands of blue grama were established inside the exclosure in 1939, and three more in 1941. The plots were clipped with heavy shears about three-fourths of an inch above the ground following a schedule that made samples available for chemical analyses at fifteen-day intervals during the growing season and at monthly intervals during the winter. Mature samples were clipped on the same dates as the samples from the plots to make possible a study of the effects of clipping on chemical composition.

Seven plots of pure stands of buffalo grass were established and were clipped in the manner set forth for blue grama. Four similar plots of Russian thistles were included in the study during the 1939 season.

All samples were brought to the laboratory at once and handled according to the procedure described by Langham, *et al.* (1943).

For the density study, duplicate unfenced plots containing one hundred square feet each were established at three intervals between terrace ridges. One set of plots was established about fifteen feet below a ridge, another set halfway between the ridges, and the third set about fifteen feet on the upper slope above the next ridge. Density readings were made at the beginning and the end of each growing season. The square foot density method as formulated by the Interagency Committee of the Western Range Survey Conference (1937) was used, and a separate estimate was made for each species studied.

The contour ridges had been established with a grader in the spring of 1938 at approximately six-inch intervals. The height and spacing of the ridges caused about one-third of the total area to be flooded during periods of heaviest rainfall.

Yearling Hereford steers or heifers were turned into the pasture in the spring as early as the grass was ready for grazing. The cattle were weighed for three days in succession to determine the initial weight and were weighed at approximate intervals of two weeks until they had utilized all the grass that should have been taken from the pasture. They were weighed three days in succession to determine the final weight. Sheep were put in the pasture when more complete utilization of weeds, particularly Russian thistles, was desirable.

Results and Discussion

The results of the chemical composition study of the relation of seasonal development and weathering to the chemical composition of blue grama,

TABLE 1.—THE RELATIONSHIP OF SEASONAL DEVELOPMENT AND WEATHERING TO THE CHEMICAL COMPOSITION OF BLUE GRAMA, BUFFALO GRASS AND RUSSIAN THISTLE[1]

Sampling date	Total moisture			Carotene mg/100 g			Crude protein %			Crude ash %			Ca. %			P. %		
	B gr[3]	B da[3]	S pe[3]	B gr	B da	S pe	B gr	B da	S pe	B gr	B da	S pe	B gr	B da	S pe	B gr	B da	S pe
May 15	55.0	56.2	71.6	32.4	27.4	32.8	13.9	14.4	25.0	11.7	9.1	—	.46	.45	4.1	.25	.28	.21
June 1	53.7	57.3	41.8	37.3	25.7	20.6	12.0	12.4	14.3	12.0	9.9	22.3	.36	.40	2.4	.26	.24	.23
June 15	51.4	57.6	66.5	16.3	23.8	10.3	9.2	11.7	13.1	12.1	11.7	18.0	.34	.38	2.1	.20	.25	.27
July 1	39.8	28.2	35.2	8.7	5.8	8.6	6.7	7.9	18.5	11.2	10.3	21.1	.31	.34	2.4	.21	.20	.25
July 15	35.2	29.4	—	10.3	10.2	—	6.3	8.6	—	12.3	10.9	—	.34	.40	—	.20	.20	—
Aug. 1	26.9	27.3	19.8	6.0	5.9	3.8	6.9	8.2	12.2	14.0	12.4	13.8	.43	.38	1.6	.19	.23	.24
Aug. 15	22.1	24.9	51.8	3.6	5.7	4.2	6.3	8.7	7.4	14.7	12.0	13.9	.31	.31	1.9	.18	.18	.21
Sept. 1	11.6	22.0	40.9	4.2	3.5	5.0	9.7	8.0	16.2	9.8	12.3	16.3	.33	.29	1.9	.15	.19	.26
Sept. 15	13.7	14.9	24.5	1.6	3.0	2.8	5.8	7.9	18.5	9.9	11.6	18.5	.25	.30	2.3	.15	.17	.20
Oct. 1	25.1	24.3	—	2.9	2.0	—	4.8	8.3	—	12.8	6.3	—	.24	.31	—	.11	.16	—
Oct. 15[2]	13.3	15.0	6.0	0.0	0.0	.7	6.7	8.2	15.1	10.8	13.7	18.3	.25	.26	2.6	.14	.15	.24
Nov. 1	9.4	6.6	5.8	—	0.0	.2	4.8	7.3	8.0	10.6	13.0	11.4	.22	.28	1.7	.11	.13	—
Nov. 15	6.1	6.2	—	—	0.0	—	5.4	8.7	—	11.0	12.7	—	.35	.31	—	.09	.12	—
Dec. 1	7.1	7.7	8.0	—	—	—	4.3	7.8	7.3	10.1	15.0	10.3	.20	.32	1.5	.07	.12	—
Jan. 1	10.1	14.2	—	—	—	—	3.9	8.2	—	11.9	14.5	—	.29	.30	—	.06	.12	—
Feb. 1	6.9	8.5	—	—	—	—	3.0	8.3	5.2	13.7	15.3	8.2	.31	.38	2.2	.06	.13	—
March 1	8.7	9.2	6.4	—	—	—	4.4	8.3	4.9	13.2	16.5	7.5	.31	.39	1.8	.06	.08	—
April 15	7.2	12.8	5.3	—	—	—	5.0	8.3	5.3	17.0	22.5	14.2	.44	.49	2.7	.13	.13	—

[1] All analyses except moisture are reported on dry weight basis—average for 3 years 1939–1941. Thistles for 1939–1940 season only.

[2] Average frost date about October 23.

[3] B gr—Blue grama. B da—Buffalo grass. S pe—Russian thistle.

buffalo grass and Russian thistles are given in table 1. A detailed report of these chemical studies is published elsewhere, Langham, *et al.* (1943). A summary of the chemical composition studies is given here so that the trend in chemical composition can be compared with the trend in livestock gains.

The moisture, protein, and phosphorous content of all three herbages dropped rapidly as the plants approached maturity, except in the case of phosphorus in Russian thistles. Calcium was quite variable throughout the season. These results are in agreement with the findings of Stanley and Hodgson (1938). The carotene content also decreased with maturity, which decrease is in agreement with the observation of Watkins (1939) for black grama and mesa drop-seed and with Atkeson, *et al.* (1937) for buffalo grass.

TABLE 2.—EFFECT OF CLIPPING ON CHEMICAL COMPOSITION OF [BLUE GRAMA AND BUFFALO GRASS. ANALYSES ARE ON DRY WEIGHT BASIS

Description	Buffalo Grass					
	Total moisture	Carotene	Crude protein	Crude ash	Ca	P
	per cent	*mg/100 g*	*per cent*	*per cent*	*per cent*	*per cent*
Av. all samples prev. cut	37.3	12.4	9.1	11.9	0.36	0.26
Ave. all samples not prev. cut	37.9	11.6	8.8	11.5	0.36	0.26
	Blue Grama					
Ave. all samples prev. cut	41.2	13.0	8.5	13.3	0.37	0.26
Ave. all samples not prev. cut	36.2	10.8	7.3	12.4	0.34	0.21

The data in table 1 reveal that buffalo grass is superior to blue grama during the winter months. The average protein content of blue grama taken after frost was 4.8% compared with 8.1% for buffalo grass. During the dormant period the phosphorus and calcium content of buffalo grass was also appreciably higher than that of blue grama.

Chemical composition studies at this station with Russian thistles stacked for hay show the hay to resemble buffalo grass in chemical composition and to be definitely superior to blue grama pasture for winter utilization.

It is interesting to note in table 2 that clipping has little effect on the chemical composition of buffalo grass. All of the nutrients were increased by frequent clipping of blue grama. Similar increases as a result of frequency of clipping were observed by Hopper and Nesbitt (1930) for a number of North Dakota grasses. It appears that small variation in chemical composition with frequency and intensity of grazing may be minor compared to the effects of climatic factors on the total yield and composition of grass.

Performance of livestock on the experimental pasture for four grazing seasons is summarized in table 3. The summary reveals that yearling Herefords grazing the pasture for an average of 129 days for four seasons made an average daily gain of 1.01 pounds and produced a yearly average of 29.3 pounds of beef per acre. The average daily gain for June was 1.47 pounds compared to .53 pound for August. When the herbage contained 13 to 25% crude protein (dry basis) and 10 to 37 milligrams of carotene per 100 grams in June, gains were high. As the protein dropped to 6 to 8% and carotene to

TABLE 3.—SUMMARY OF PERFORMANCE OF LIVESTOCK ON 32-ACRE-EXPERIMENTAL PASTURE, 1939–1942

Item	1939	1940	1941	1942	4 yr. ave.
Number of cattle	11 heifers	4 steers	6 heifers	5[b] heifers	6.5
Date weighed in	5–27	6–5	5–16	4–16	5–16
Date weighed out	10–22	9–19	10–15	9–3	9–30
Total grazing days (cattle)	1598[a]	424	912	796[b]	932
Length of grazing season	148	106	152	110	129
Initial weight lbs.	623	569	501	564	—
Final weight lbs.	761	689	643	695	—
Total gain lbs.	1518	480	852	912	940
Average daily gain lbs.	0.95	1.13	0.93	1.15	1.01
Pounds gain per acre	47.4	15.0	26.6	28.5	29.3
Other livestock (cattle days equivalent)	—	525[c]	136[d]	657[e]	—

[a] Two tons sudan grass hay fed heifers in Sept. and Oct., 1939—one heifer weighed in 6-26-39.
[b] Six extra steers on pasture for 43 days—April 16 to May 12 and from June 16 to July 1, gained 257.4 pounds.
[c] Sheep (118) for 35 days—from June 8 to July 11.
[d] Horses (3) May 16 to July 1.
[e] Sheep (175) June 6 to July 5.

3 to 6 milligrams in August, average livestock gains were correspondingly low. These figures indicate that gains are related to chemical composition.

The above gain per acre and the seasonal trends in gain are in agreement with the results of Sarvis (1941) for North Dakota and Williams and Post (1941) for Montana. Lower average daily gains than reported by these other investigators are probably due to over-grazing, especially during the 1939 season.

An interesting side light on the cattle grazing results is the fact that the carrying capacity of the pasture was nearly doubled during the 1940 and the 1942 grazing seasons by pasturing sheep to utilize surplus Russian thistles in 1940 and rapidly growing grass in 1942 that the cattle could not fully utilize.

Density estimates of the vegetation for the species studied, taken spring and fall, are shown in table 4. It will be noted that there was a great increase

in density on the area which was flooded above the terrace ridges. There was also a considerable decrease in the density of grass in 1940 on the part not flooded as compared with the density of the previous year. This decrease was apparently due to extremely dry weather during the late summer of 1939 and to the possibility that the rate of stocking was not decreased

TABLE 4.—ESTIMATED DENSITY OF VEGETATION ON RIDGED PASTURE[1]

Type of grass and space observed	May 1939	Oct. 1939	May 1940	Sept. 1940	June 1941	Oct. 1941	May 1942	Oct. 1942
Buffalo grass								
Below ridge	1.62	2.21	.17	.25	.21	.79	.46	2.37
Middle of interval	2.58	5.00	.25	.54	.13	1.83	1.25	6.33
Above ridge	4.67	7.58	6.75	8.25	10.33	12.12	8.12	11.71
Blue grama								
Below ridge	4.96	3.58	1.25	1.12	.79	1.37	1.04	1.54
Middle of interval	4.50	2.37	.81	.78	.25	1.17	.75	2.87
Above ridge	6.62	4.87	4.25	3.75	2.08	2.96	1.75	4.67
Russian thistle								
Below ridge	.96	1.41	2.21	3.25	.87	2.25	—	—
Middle of interval	1.50	.75	.71	2.67	.25	1.83	—	—
Above ridge	.21	.41	—	—	—	—	—	—

[1] All figures shown are averages of duplicate readings made in three intervals between ridges The square-foot method was used in estimating density of vegetation.

sufficiently to allow for the extremely adverse weather conditions. It can be seen that there was a very rapid recovery of grasses, especially buffalo during the wet seasons of 1941 and 1942.

Although Russian thistles were prominent in dry seasons, the grasses tended to crowd them out during the wet seasons of 1941 and 1942.

Summary

Beginning in May, 1939, samples of buffalo grass, blue grama and Russian thistles were taken bi-monthly from fenced square-meter plots in a 32-acre terraced pasture for chemical composition study. Density readings were taken spring and fall from unfenced plots. Yearling Herefords grazing the pasture were weighed every fifteen days during the grazing season. Sheep were used to utilize weeds and surplus grass in 1940 and 1942 respectively.

Moisture, crude protein, carotene and phosphorus were high in the three herbages during the early stages of growth but decreased rapidly as the plants approached maturity, usually during July. Buffalo grass proved to be superior to blue grama for winter pasture, and its chemical composition was affected less by clipping.

Yearling Herefords grazing the pasture for an average of 129 days for 4 seasons made an average daily gain of 1.01 pounds and produced a yearly average of 29.3 pounds of beef per acre. The average daily gain for June was 1.47 pounds compared to .53 pound for August. Gains were high when the protein and carotene content of the herbage was high in June and correspondingly low in August. Sheep were used early in the seasons of 1940 and 1942 to utilize more completely weeds and surplus grass. The use of sheep added materially to the grazing capacity of the pasture.

There was a great increase in the density of buffalo grass above the terrace ridges. In the dry season of 1940 there was a considerable decrease in density of grass on unflooded areas below the terraces. Although Russian thistles were prominent in dry seasons, the grasses tended to crowd them out during the wet seasons of 1941 and 1942.

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THE PREPARATION OF MUSCULAR TISSUES FOR HISTOLOGICAL STUDY[1]

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A REVIEW of the studies which have been made on the histology of meat at Purdue University and by other investigators has revealed that entirely satisfactory techniques for the preparation of muscular tissues for microscopic study have not been adequately developed. Muscular tissues which have been described as representative of the normal microscopic anatomy of the living animal have, almost without exception, shown evidences of excessive shrinkage of the individual muscle fibers and obviously do not illustrate the structure of muscle in the intact animal.

If sound conclusions are to be drawn concerning the relationship of skeletal muscle histology with such variables as species, age, sex, plane of nutrition and influence of freezing upon meat quality, it would seem advisable to adopt carefully standardized methods. The objective of this paper, therefore, is to outline the procedures which have been found most successful in our laboratory.

Procedures

Fixation

By definition fixation is the process of preserving the normal gross and microscopic structures of organs, tissues and cells. It is probably the most important single step in histological work, for unless the cellular characteristics of the living tissue are preserved in permanent form the final results will be unsatisfactory. The time which elapses between the death of an animal and the complete fixation of its tissues is of vital importance. The most critical histologists endeavor to place the desired tissues in fixing reagents before the animal's heart ceases to beat and fixatives are often injected directly into the circulatory system of the intact animal. Refinements and speed of this sort are obviously impossible in meat animals, but investigators should attempt to fix tissues before experimental animals leave the killing floor (within 1–2 hours after slaughter). Although whole organs are often prepared for microscopic study, tissue size and quality of fixation are highly correlated. It is recommended that tissue blocks of not more than 8 mm. in diameter be used for routine studies.

Solutions of single substances, e.g., alcohol, acetic acid, chloroform, picric acid or bichloride of mercury are seldom used, but give excellent results in

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combination with other reagents. Since some chemicals (mercuric chloride and picric acid) tend to shrink tissues, this reaction is usually overcome by reagents which have a swelling effect (acetic acid). Bouin's, Tellyesnicky's and Zenker's fluids, Dioxan combinations, and absolute alcohol, although excellent for some tissues, did not give satisfactory results with striated muscle. The following fixatives were found most reliable:

Alcohol—Acetic Acid

Glacial acetic acid—1 part
Absolute ethyl alcohol—3 parts
Fix 10 hours exactly

Carnoy's Chloroform-Alcohol—Acetic Acid

Chloroform—3 parts
Absolute ethyl alcohol—6 parts
Glacial acetic acid—1 part
Fix 10 hours exactly.

Formalin—Alcohol—Acetic Acid

50% Ethyl alcohol—90 cc.
40% Formalin—5 cc.
Glacial acetic acid—5 cc.
Fix 4–5 hours—no longer.

Formalin

Although 10% formalin is widely used in routine work, it is not considered satisfactory for the preservation of critical cellular detail. Since it is one of the easier fixatives to use and since muscle fixed in it is usually easily cut, it can be used as a supplementary fixing agent with some success. We have found a fixation time of 10 hours satisfactory with 10% formalin.

The Altman Freezing-Drying Method

The Altman technique, which was developed in its modern form by Gersh (1932), has been applied to meat by Koonz and Ramsbottom (1938). Fixation is accomplished by freezing small blocks of tissue in chilled pentane at $-131°$ C. or iso-pentane at $-195°$ C. and dehydration is secured by placing the frozen tissue in a vacuum desiccator and dehydrating under vacuum at $-30°$ to $-50°$ C. for 24 hours. Simpson (1941) in an extensive study of the application of the method for various types of tissue concluded that the preservation of cytological details in the outer layer of tissue frozen at $-195°$ C. was superior to that obtained by chemical fixation. There was a definite relationship between tissue size and the quality of fixation. Simpson gives no tissue size limits, since the density or nature of the tissue is also a variable, but a photomicrograph of a section of liver approximately 3 mm. thick shows 3 very definite zones of fixation. It would appear, then, that

tissue size, speed and method of freezing would need to be carefully controlled in order that artefacts not be introduced. From the theoretical standpoint the freezing-drying method has some very definite advantages, especially in the fixation of tissues that have already been frozen. However, the apparatus required limits this method to laboratory use and practically prohibits its application for fresh tissue on the killing floor.

Dehydration and Clearing

Dehydration is the process of water removal from the tissue and is normally carried out by the replacement of the water with a solvent, usually alcohol. The composition of the fixative will determine the nature of the dehydrating agent, the objective being to gradually replace the tissue water with some other substance in order that the violent diffusion of greatly different substances not damage the tissue structures. Since, following dehydration, the tissues must be infiltrated with paraffin or collodion, the dehydrating agent must be replaced by paraffin or collodion solvents. This was formerly accomplished by placing the tissues in xylol, chloroform, benzol or other hydrocarbons, but is now usually brought about by the use of butyl alcohol or dioxan. Some tissues can be passed directly from fixatives to dioxan and then into paraffin, but we have not been able to secure satisfactory results with skeletal muscle by this method. We have found butyl alcohol very useful as a dehydrating, clearing and infiltrating agent and the recommended time intervals will be subsequently presented.

The time and strength of dehydrating agents is of vital importance. The higher alcohols and other solvents tend to harden tissues, and eventually cause difficulties in cutting. The freezing-drying method eliminates the use of chemicals during fixing and dehydration but the denaturation of such tissues by placing them in redistilled absolute alcohol for approximately one week before imbedding in paraffin (Simpson, 1941) introduces an additional and lengthy procedure.

In these studies the following reagents and procedures were used during dehydration and clearing:

Following fixation for 10 hours in alcohol—acetic acid or Carnoy's chloroform-alcohol-acetic acid:

Wash

95% ethyl alcohol—2 to 3 changes over night. Until acetic acid odor disappears.

Dehydrate

½-95% ethyl alcohol and ½ butyl alcohol for 1 to 2 hrs.
Absolute butyl alchohol, two changes during a 12 hour period.

Following 4 to 5 hours fixation in Formalin-Alcohol-Acetic Acid or 10 hours fixation in 10% Formalin:

Wash

70% ethyl alcohol—2 changes over night.

Dehydrate

⅔—70% ethyl alcohol and ⅓ butyl alcohol for 1 to 2 hrs.
⅓—95% ethyl alcohol and ⅔ butyl alcohol for 1 to 2 hrs.
Absolute butyl alcohol, two changes during a 12 hour period.

Infiltration

The replacement of the clearing agent (butyl alcohol) in the tissue with melted paraffin is called infiltration. If the tissue has not been thoroughly dehydrated and cleared it is impossible for paraffin to be dispersed evenly through it. It is difficult if not impossible to cut satisfactory sections of poorly infiltrated tissues. Time and temperature are very important during infiltration. As a general rule the tissue should be left in paraffin only for the time required for paraffin to completely saturate it. The paraffin should be maintained at a temperature sufficient to keep it in the liquid state but no higher. Tissues which are allowed to remain in the paraffin oven for excessive periods of time or at high temperatures become hard and brittle, are difficult to cut and are changed in microscopic appearance.

The following procedures have proved satisfactory:

Infiltrate

2 hours in butyl mush (½ butyl alcohol and ½ paraffin)
2 hours in 40 to 52°C.m.p. paraffin. (1st change)
2 hours in 40 to 52°C.m.p. paraffin. (2nd change)
2 hours in 56 to 58°C.m.p. paraffin. (3rd change)
Imbed in 56 to 58°C.m.p. paraffin.

Cutting

Tissues which have been properly fixed, dehydrated, cleared and infiltrated can usually be cut on a rotary microtome in sections from 6 to 12 microns in thickness. Cross sections of the muscle fibers are usually more satisfactory for purposes of study than are longitudinal sections although in some instances both planes of section are desirable. The individual skill of the technician is of more value than a fixed method of procedure during cutting. The use of a sharp knife and the operation of the microtome in a room warm enough to allow satisfactory ribboning of the sections will usually yield tissues of good quality.

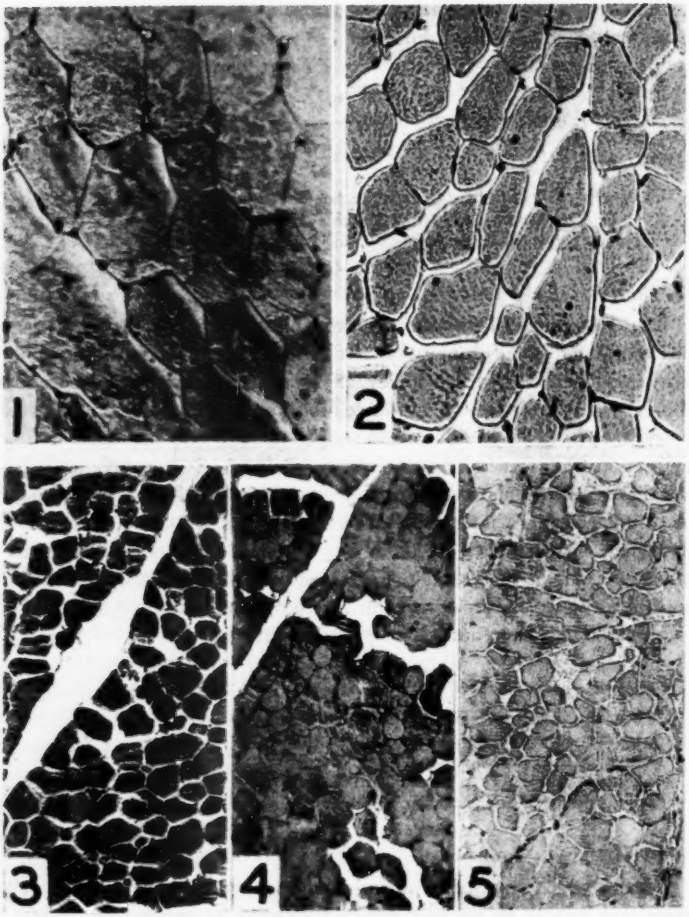

Figure 1. Normal beef muscle fixed in Alcohol-Acetic Acid within 1–2 hours after slaughter. Note the close packing of the muscle fibers. 220×.

Figure 2. Beef muscle ripened 21 days and fixed in 10% Formalin. Observe the shrinkage and separation of the fibers. 220×.

Figure 3. Beef muscle ripened 21 days, frozen and stored for 15 months and fixed in 10% Formalin. The muscle fibers show shrinkage and separation and connective tissue is not prominent. 100×.

Figure 4. The same as Figure 3 but fixed in Formalin-Alcohol-Acetic Acid. Observe the close arrangement of the fibers. 100×.

Figure 5. The same as Figure 3 but fixed in Chloroform-Alcohol-Acetic Acid. There is less shrinkage than in 10% Formalin and connective tissue is apparent. 100×.

The Influence of Fixation Methods Upon the Microscopic Anatomy of Meat

The cross section of a piece of beef loin fixed within two hours after slaughter in Alcohol-Acetic Acid is shown in figure 1. The individual muscle fibers do not show the separation and obvious shrinkage which is commonly observed in studies of the histology of fresh and frozen meat. A section of beef muscle ripened at 32 to 34° F. for 21 days is shown in figure 2. This tissue was prepared in our laboratory several years ago, was fixed in 10% Formalin and shows marked shrinkage and separation of the individual muscle fibers in contrast to the microscopic structure shown in figure 1. Since meat loses water during storage one might logically expect some shrinkage of the muscle fibers during ripening. The changes demonstrated in figure 2, however, appear to be greatly accentuated by fixation in 10% Formalin.

Numerous studies on the influence of freezing upon the histology of meat have been published. It is not within the scope of this paper to discuss the influence of freezing upon the microscopic anatomy of striated muscle but to describe the influence of method of preparation upon the histological appearance of tissue. A small section of a beef loin which had been ripened for 21 days at 32 to 34° F., frozen at −26° F. and stored at 0 to 5° F. for 15 months, was removed, divided into 3 equal portions and fixed in 10% Formalin, Formalin-Alcohol-Acetic Acid and Chloroform-Alcohol-Acetic Acid. The microscopic structure of the tissues fixed in these reagents are shown in figures 3, 4, and 5. The muscle fibers in figure 3 (10% F) have undergone definite shrinkage and separation, and there is little evidence of connective tissue between the bundles of muscle fibers. The tissue fixed in FAA (figure 4) shows, in selected areas of the photomicrograph, practically no shrinkage or separation of the fibers, although the fibers tend to be separated into groups. Connective tissue between bundles is not apparent and the muscle fibers did not stain as readily as following 10% Formalin. The same tissue fixed in CAA is shown in figure 5. This section is intermediate in appearance between figures 3 and 4. There is some separation of the muscle fibers but less than in figure 3. Connective tissue between the muscle bundles is evident. These photomicrographs demonstrate the marked differences which may occur within the same tissue when different techniques are used and emphasize the necessity of preparing tissues for microscopic study by at least three different methods. Artifacts occur in the hands of the most careful workers and it would seem that the best insurance against their undetected occurrence is the use of triplicate samples of meat.

Conclusions

The procedures for the preparation of beef muscle for microscopic study

following fixation in Alcohol-Acetic Acid, Carnoy's Alcohol-Acetic Acid, Formalin-Alcohol-Acetic Acid and 10% Formalin are outlined.

The desirability of utilizing at least three different methods of fixation in the preparation of beef tissues for histological examinations is stressed.

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INDUCTION OF ESTRUS AND OVULATION IN EWES DURING THEIR ANESTRUS SEASON

A. H. FRANK, *Associate Veterinarian*, AND AARON APPLEBY, *Junior Veterinarian, Animal Disease Station, U. S. Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md.*

MOST sheep have a limited breeding season in the fall and early winter with a rather long anestrous period between breeding seasons. Some experimental work has been conducted in attempts to bring ewes in estrus during this anestrous period in order to spread the breeding season or in attempts to obtain two lamb crops per year. Available results indicate that a majority of the ewes treated with certain sex hormones may be brought into estrus, but many of these fail to become pregnant.

Polovceva and Fomenko (1940) injected two groups of ewes with pregnant mare's serum. One group was given two injections 16 days apart. Twenty per cent of this group came into estrus and seven per cent lambed. The other group received three injections given on the 1st, 15th and 16th days. Forty-eight per cent of this group came into estrus and 20.5 per cent lambed. August (1941) produced estrus in anestrous ewes with 10,000 and 15,000 international units of estradiol benzoate. Of 4,000 animals treated, 72.5 per cent mated and 43.1 per cent lambed. Hammond, Hammond and Parkes (1942) state that gonadotropic extracts of horse-pituitary gland, of pregnant mare's serum and of urine of pregnant women, as well as estrogenic substances, will induce ovulation in anestrous sheep. They found that a normal number of ova were shed regardless of the amount of hormone given. These authors have given a comprehensive review of the literature on the use of hormones in sheep. D. H. Wonder, of the Cutter Laboratories, through correspondence, relates that in a number of large-scale range experiments with the use of "gonadin"[1] in sheep their results have been quite variable. In some instances an exceptionally large number of lambs were born with a considerable shortening of the lambing period, but in other trials no effect could be traced to the use of the hormone.

For practical purposes, aside from increasing the breeding efficiency of sheep, the effects of the hormones upon the ewe during the anestrous period may serve as a source of information for their clinical application in other species. If the estrous cycle, simulating that during the breeding season, could be initiated in ewes during their anestrous period, then it should be possible to breed them with the same efficiency as at their normal mating time. The gonadotropic hormones secreted by the anterior pituitary gland activate the gonads which, in turn, initiate sexual activity. Large amounts

[1] The commercial name given to the gonadotropic hormone derived from pregnant mare's serum, by the Cutter Laboratories, Inc.

of estrogenic hormone depress the activity of the pituitary gland, but small amounts stimulate it.

In an attempt to initiate the normal estrous cycle in anestrous ewes, it might be expected that the minimum amount, or less, of stilbestrol required to induce estrous should stimulate the pituitary body into increased activity. A sufficient response from this gland should re-establish the normal estrous cycle.

To throw further light on the possibilities of developing a method for increasing lamb production, experimentation was made during the summer of 1942 on the efficacy of two sex hormones which are known to be involved in normal reproduction: that found in pregnant mare's serum, and diethylstilbestrol.

Material and Methods

The synthetic estrogenic preparation, diethylstilbestrol[2] (stilbestrol), the potency of which was measured in milligrams, was obtained in oil solution. The other hormone, a gonadotropic product derived from pregnant mare's serum (gonadin)[1] was standardized to contain 50 rat units per cubic centimeter. All injections were given subcutaneously. The time of the first injection of stilbestrol or of P.M.S. was called the first day, and subsequent injections were counted from that day.

Fifty ewes and eleven rams were selected for this work. Previous breeding records on these sheep were not available. Semen was collected from the rams at frequent intervals to check its quality.

Data and Discussion

The minimum dosage of stilbestrol necessary to induce estrus was determined by the injection of various quantities (table 1). After injections were made, the ewes were teased with a ram each day to determine the presence and duration of estrus. There was quite a variation among individual ewes in their response to stilbestrol. A dosage of 2 mg. on the first day and 1 mg. on the second day was effective in inducing estrus in eleven of twelve animals. In all cases estrus occurred in 96 hours or less after the first injection. A few cases responded in 24 hours, but the majority of the ewes responded around 48 hours. The duration of estrus averaged from two to three days.

In six of the ewes, in five of which estrus had been induced, the injections were discontinued for 20 days. During this time, the ewes were teased with the ram each day to determine if estrus would occur spontaneously. Spon-

[2] The diethylstilbestrol was very generously furnished by the Winthrop Chemical Company, Inc., New York City, and the gonadin by the Cutter Laboratories, Inc., Berkeley, California.

TABLE 1.—INDUCTION AND DURATION OF ESTRUS AT STATED INTERVALS FOLLOWING SUBCUTANEOUS INJECTIONS OF DIETHYLSTILBESTROL

Dose in Mgs.	No. of ewes injected	24 hours			48 hours			72 hours			96 hours			120 hours			144 hours			Estrus induced in—	
		Estrus induced	Still in estrus	Anestrus	Estrus induced	Still in estrus	Anestrus	Estrus induced	Still in estrus	Anestrus	Estrus induced	Still in estrus	Anestrus	Estrus induced	Still in estrus	Anestrus	Estrus induced	Still in estrus	Anestrus	No. of ewes	% of ewes
1	8	0	0	8	1	0	7	0	0	8	0	0	8	0	0	8	0	0	8	1	12
2	12	1	0	11	3	1	8	0	3	9	0	0	12	0	0	12	0	0	12	4	33
2½	6	0	0	6	1	0	5	1	0	5	0	0	6	0	0	6	0	0	6	2	33
3	12	3	0	9	3	3	6	1	5	6	1	1	10	0	1	11	0	1	11	8	66
2+1[1]	12	3	0	9	6	3	3	1	7	4	1	5	6	0	2	10	0	0	12	11	92

[1] Two mg. given one day followed by one mg. on the next day.

taneous estrus did not occur in any of these ewes. It was therefore assumed that ovulation was not induced. Laparotomies, for the purpose of determining the response of the ovaries to the injections, were not performed.

Pregnant mare's serum was next used to stimulate the ovaries directly. Five ewes (group 1, figure 1) were injected with 5 to 10 cc. of P.M.S. and killed on the third day. One or two follicles in the ovaries of each ewe appeared to be stimulated slightly as judged by their size, but ovulation had not occurred.

Six ewes (group 2, figure 1) were each given a single injection of 5 cc. of P.M.S. All of these ewes were "force" bred on the 2nd, 4th and 5th days. One ewe came into estrus and was bred on the 6th day. On the 8th day, laparotomy was performed on two ewes. Ovulation had occurred in both animals. Pregnancy was not induced in any case.

It was thought that the dosage of P.M.S. might have been too small to induce estrus accompanying ovulation. Four ewes (group 3, figure 1) were given larger doses. One ewe received 10 cc. at a single injection and another received 5 cc. on each of two successive days. Laparotomies were performed on the 11th day. Three corpora lutea were found in each case. One ewe received 5 cc. on the 1st day and 20 cc. on the 2nd day. Laparotomy was performed on the 8th day. One ovary contained one corpus luteum and five enlarged follicles, and the other ovary contained three enlarged follicles. The follicles were similar in size to those at the time of ovulation. The 4th ewe was given 10 cc. on each of two successive days. When examined on the 8th day by laparotomy, one ovary contained seven corpora lutea and the other ovary one corpus luteum. Estrus was not induced in any of these ewes.

As the larger amounts of P.M.S. given at one time failed to induce estrus, three ewes (group 4, figure 1) were given a smaller daily dosage. The initial dose was 2½ cc. each. Beginning on the 3rd day, each was given 1 cc. daily. In two ewes, daily injections were continued for six days, but estrus was not induced. One of these had ovulated when laparotomy was performed. Another ewe was given daily injections for three days at which time she came into estrus and was bred. Ovulation was induced, as was found later upon laparotomy, but she did not become pregnant.

Breeding experiments have been reported wherein the majority of anestrous ewes were brought into estrus and bred by giving two injections of P.M.S. 16 days apart. Estrus and ovulation followed the second injection, and many ewes become pregnant when bred at that time.

Six ewes (group 5, figure 1) were each given an initial injection of 5 cc. of P.M.S. This was followed by a second injection on the 16th day, three ewes receiving 1 cc. each and three 5 cc. each. After the 2nd injection, four ewes came into estrus and were bred. Laparotomies were performed on five of the

ewes, including the two that did not come into estrus, one of which had received 1 cc. and the other 5 cc. Each ewe had fresh corpora lutea that had been induced after the second injection. The 6th ewe, which was not operated upon, later proved to be pregnant from services during that estrus period. No other pregnancies were produced.

Estrus was not induced in all of the ewes in any of the above groups, but ovulation had occurred in all ewes on which laparotomy was performed.

It is known that estrus normally is induced by the action of estrogenic hormone, and at normal estrus it is assumed that this activating hormone is secreted by the developing follicle. The follicles induced by P.M.S. apparently failed to secrete sufficient hormone. In such cases it was assumed that the injection of small doses of stilbestrol might stimulate the sexual apparatus sufficiently for the hormone of the follicle to induce estrus in proper timing with ovulation. Therefore, the minimum amount, or less, of stilbestrol necessary to induce estrus in the majority of ewes was used in different combinations with P.M.S. in the following groups.

Six ewes (group 6, figure 1) were each given simultaneous injections of 5 cc. of P.M.S. and 2 mg. of stilbestrol and, on the 2nd day, 1 mg. of stilbestrol. Estrus was induced in 5 ewes. They were all bred on the 3rd and 4th days, and those in estrus were bred again on the 5th day. None of these ewes became pregnant. Laparotomies were not performed.

Ten ewes (group 7, figure 1) were given 5 cc. of P.M.S. on the first day and three of the ewes were given 1 mg. of stilbestrol on the 3rd day, but estrus was not induced. The three ewes were force-bred on the 6th and 7th days. Laparotomies, performed on two of the ewes, showed that each had ovulated. Six of the ewes received 2 mg. and 1 mg. of stilbestrol on the 2nd and 3rd days respectively. They were all force-bred on the 3rd day. Five came into estrus on the 4th day and were bred, the sixth one being force-bred. All were bred again on the 5th day, and two on the 6th day. Laparotomies were performed on two of the ewes. The one that did not come into estrus had ovulated, and one that did come into estrus had not ovulated. The last ewe of this group was given 1, 1, ½ and 1 mg. of stilbestrol on the 3rd, 4th, 5th and 6th days respectively, and came into estrus on the 6th day. A second 5 cc. dose of P.M.S. was given on the 7th day and she was in estrus on the 8th and 10th days. She was not teased on the 7th and 9th days. Laparotomy was performed on the 11th day. Ovulation had occurred on the 6th day as judged by the appearance of the corpus luteum. A second laparotomy was performed on the 21st day but adhesions prevented the examination of one ovary. The available ovary contained the corpus luteum present at the previous operation, but no other changes were observed. None of these ewes became pregnant.

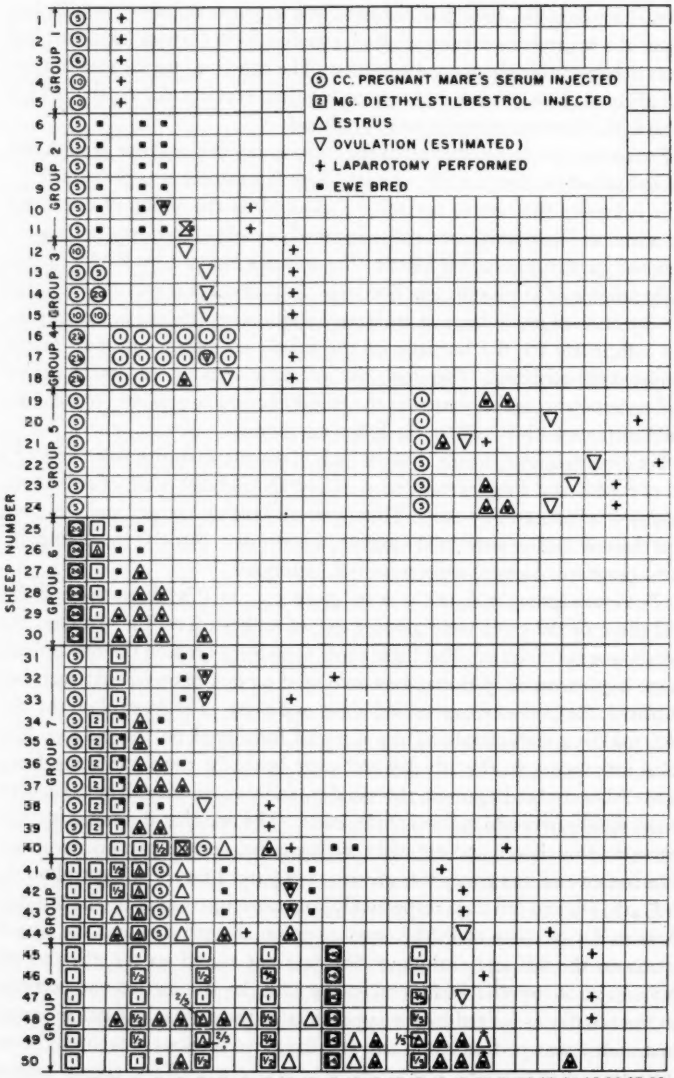

Figure 1. Time of injection, estrus, and ovulation in sheep.

Four ewes (group 8, figure 1) were each given a subminimal dose of stilbestrol daily until estrus was induced. Two ewes received 1, 1, ½ and 1 mg. each on successive days and came into estrus on the 4th day. The other two ewes received 1 mg. each on two successive days and came into estrus on the 3rd day. These latter were given 1 mg. again on the 4th day. Each of the four ewes was given 5 cc. of P.M.S. on the 5th day and remained in estrus on the 6th day. They were all bred on the 8th day, one rebred on the 10th, all on the 11th, and two on the 12th day. Laparotomies were performed on the first three ewes on the 18th and 19th days. Ovulation had been induced in two of them. The last ewe was also in estrus on the 8th day. A laparotomy was performed on the 9th day, but ovulation had not occurred. She was not examined for estrus on the 9th and 10th days, but was in estrus on the 11th day. A second laparotomy was performed on the 23rd day and ovulation had occurred. From the appearance of the corpus luteum, ovulation was judged to have taken place a few days later than in the two aforementioned ewes. In all of these ewes, ovulation was judged to have occurred several days after the cessation of estrus. None of the ewes became pregnant.

The above combinations of injections did not induce estrus and ovulation to simulate normal timing. A final group of six ewes (group 9, figure 1) was given a small dose of stilbestrol ranging from ⅕ mg. to 1 mg. per injection every 3rd day for five consecutive injections. On the 13th day 5 cc. of P.M.S. were given simultaneously with the stilbestrol. A final dose of stilbestrol was given on the 17th day. The stilbestrol was given at these intervals to prevent its producing a cumulative effect. However, estrus did occur intermittently in three ewes, but ovulation was not induced in any of them. These ewes were bred, with few exceptions, while they were in estrus, but did not become pregnant. Estrus was not induced in the other three ewes. Laparotomy revealed that ovulation had occurred in one of them.

Summary and Conclusions

Estrus may be induced in ewes during their anestrus season by the injection of diethylstilbestrol (stilbestrol). Two milligrams on the first day and one milligram on the second day induced estrus in eleven of twelve animals. Spontaneous estrus did not occur within 20 days after it had been induced with the hormone.

Ovulation was proved by laparotomy to be induced in all of thirteen ewes that had received one or more injections of pregnant mare's serum. Estrus rarely occurred from a single injection or daily injections of P.M.S., but four of six ewes given two doses 16 days apart came into estrus following the second injection, and one became pregnant.

The time factor in the induction and duration of estrus by stilbestrol was

neither increased nor decreased by P.M.S., but stilbestrol did appear to have an inhibitory effect on the action of gonadin, since in 7 of 15 instances ovulation was not induced when these substances were injected in combination. When stilbestrol was injected in minimal quantities, or less, necessary to induce estrus, it did not enhance the action of P.M.S. sufficiently to induce estrus timed normally with ovulation.

Within the limits of cited experimentation, stilbestrol, either alone or in combination with pregnant mare's serum, does not appear to induce in anestrous sheep the definite hormonal stimulation of normal estrus necessary for successful fecundation.

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NEWS AND NOTES

The thirty-sixth annual meetings of the American Society of Animal Production will be held at the Hotel Sherman in Chicago on November 30 and December 1, 1943. Titles and abstracts of papers to be presented at these meetings must be in the hands of the Secretary, Dr. A. D. Weber, Kansas State College, Manhattan, Kansas, by September 15. Titles received after this date cannot be included in the program and abstracts cannot be published. The abstracts must not exceed 250 words (usually one page or less, double spaced). *Please check items 5 and 6 of "Information for Contributors."* Chairmen of the sectional meetings are:

Animal Breeding	G. E. Dickerson, Ames, Iowa
Beef Cattle	Bradford Knapp, Jr., U.S.D.A.
Dairy Cattle	C. B. Bender, New Jersey
Extension	H. G. Zavoral, Minnesota
Horses and Mules	L. H. Blakeslee, Michigan
Meats	R. S. Glasscock, Florida
Nutrition	J. K. Loosli, New York
Pasture	R. H. Lush, Washington, D. C.
Sheep and Wool	W. G. Kammlade, Illinois
Swine	M. L. Baker, Nebraska
Teaching	F. L. Bentley, Pennsylvania

A canvass of the members of the Western Section of the American Society of Animal Production revealed that a substantial majority were of the opinion that it would be inadvisable to convene the annual meeting this summer. Acting on this information, the President, Professor J. H. Knox of the New Mexico Station, wishes to announce that the regular meeting will not be held this year. It is suggested that the present officers continue in office until it is deemed advisable to call a regular meeting. Members are welcome to indicate their reaction to this proposal and also to the matter of resumption of the annual meetings.

Proceedings of the second annual conference of the New Zealand Society of Animal Production have appeared in mimeographed form and extend to 99 pages. The sixteen articles, in keeping with the agricultural conditions of New Zealand, mainly concern dairy cattle, sheep, pastures, and animal health. But there are also two articles on digestibility, one on dehydrated meat, another on the nutritive value of New Zealand lamb and mutton, and one on fisheries. Criticisms and discussions which followed the papers are included. The list of members includes 79 names. Secretary-Treasurer is Mr. A. H. Ward, New Zealand Dairy Board, Invicta House, Johnston Street, Wellington, New Zealand.

"A 'Preliminary Report on Pasture Investigations Technique' prepared by a joint committee representing the societies of Agronomy, Animal Production, and the Dairy Science Association, has been printed in the April issue of the 'Journal of Dairy Science.' Those who do not have access to this Journal may obtain a reprint by writing to The National Fertilizer Association, 616 Investment Building, Washington, D. C."

Dr. L. A. Maynard, President of the A.S.A.P. is serving this summer as nutrition expert on a joint committee sponsored by the United States and the United Kingdom Governments to make a study of food consumption levels and nutrition needs in the two countries.

Bradford Knapp, Jr., who for the past 7 years has been with the U.S.D.A. beef cattle office, Beltsville, Maryland, has been transferred to the U. S. Range Livestock Experiment Station, Miles City, Mont.

Professor A. E. Cullison of Mississippi State College has recently entered the Army as Second Lieutenant.

F. W. Bealle, extension animal husbandman, Oklahoma A. & M. College, has resigned his position to become manager of a purebred cattle ranch at Steamboat Springs, Colo.

T. A. Ewing, extension animal husbandman, University of Missouri, has resigned to become county agricultural agent of Boone Co., Missouri, with headquarters at Columbia. Mr. Ewing succeeds Wendell Holman who has accepted a position with the National Live Stock and Meat Board, Chicago.

Fred H. Leinbach, head of the animal husbandry department, University of Maryland,

took leave on June 1 to become director of the newly organized National Livestock Conservation Program with headquarters at 59 E. Van Buren St., Chicago.

Marvin Kroger has been appointed assistant animal husbandman and instructor in animal husbandry at the New Mexico A. & M. College.

Dale Snodgrass, assistant extension animal husbandman, N. C. College of Agriculture, has resigned to accept a position as agricultural agent with the Atlantic Coast Line R.R. with headquarters at Jacksonville, Fla. Mr. Snodgrass succeeds C. D. Swaffar who recently became a field representative of the American Shorthorn Breeders Assn. with headquarters at Kansas City, Mo.

D. R. Forrester, recently with the Farm Security Administration, and formerly with the Bureau of Animal Industry, has accepted an assignment to Costa Rica, C. A., with the Office of Inter-American Affairs.

W. E. Connell, who has been with the Soil Conservation Service has been put in charge of feeding research in beef and dairy cattle, sheep and hogs at the Colorado Agricultural College.

R. H. Black, asst. in animal husbandry, W. Va. University, is now on leave for military service.

J. I. Miller has assumed responsibility of the meats laboratory at Cornell University succeeding R. B. Hinman who is now on the inactive list due to illness.

Charles Hobbs, former associate professor of animal husbandry, Oklahoma A. & M. College, has joined the extension staff at Cornell University to work with adults and juniors mainly in the beef cattle field.

Thomas G. Paterson, formerly a member of the instruction staff in animal husbandry, University of Minnesota, and more recently manager of different pure-bred cattle enterprises, has been made Secretary of the Texas Hereford Association.

Milton E. Miller, formerly manager of the Killbuck Farm in Ohio, has been made assistant extension animal husbandman at the University of Maryland.

T. B. Keith, asst. professor of animal husbandry, Pa. State College, has been commissioned a captain in the food and nutrition section of the Sanitary Corps, U. S. Army. A. L. Anderson of Iowa State College has received a similar assignment.

J. R. Neale, recently asst. regional director, Farm Security Administration at Denver, Colo., and formerly extension animal husbandman, University of Wyoming, has accepted an assignment as animal husbandry specialist in a food production project for the rubber production program in the headwaters of the Amazon River in South America for the Office of Inter-American Affairs.

R. R. Snapp, animal husbandry department, University of Illinois, has been commissioned a lieutenant colonel in the U. S. Army and his work at the institution taken over by F. C. Francis.

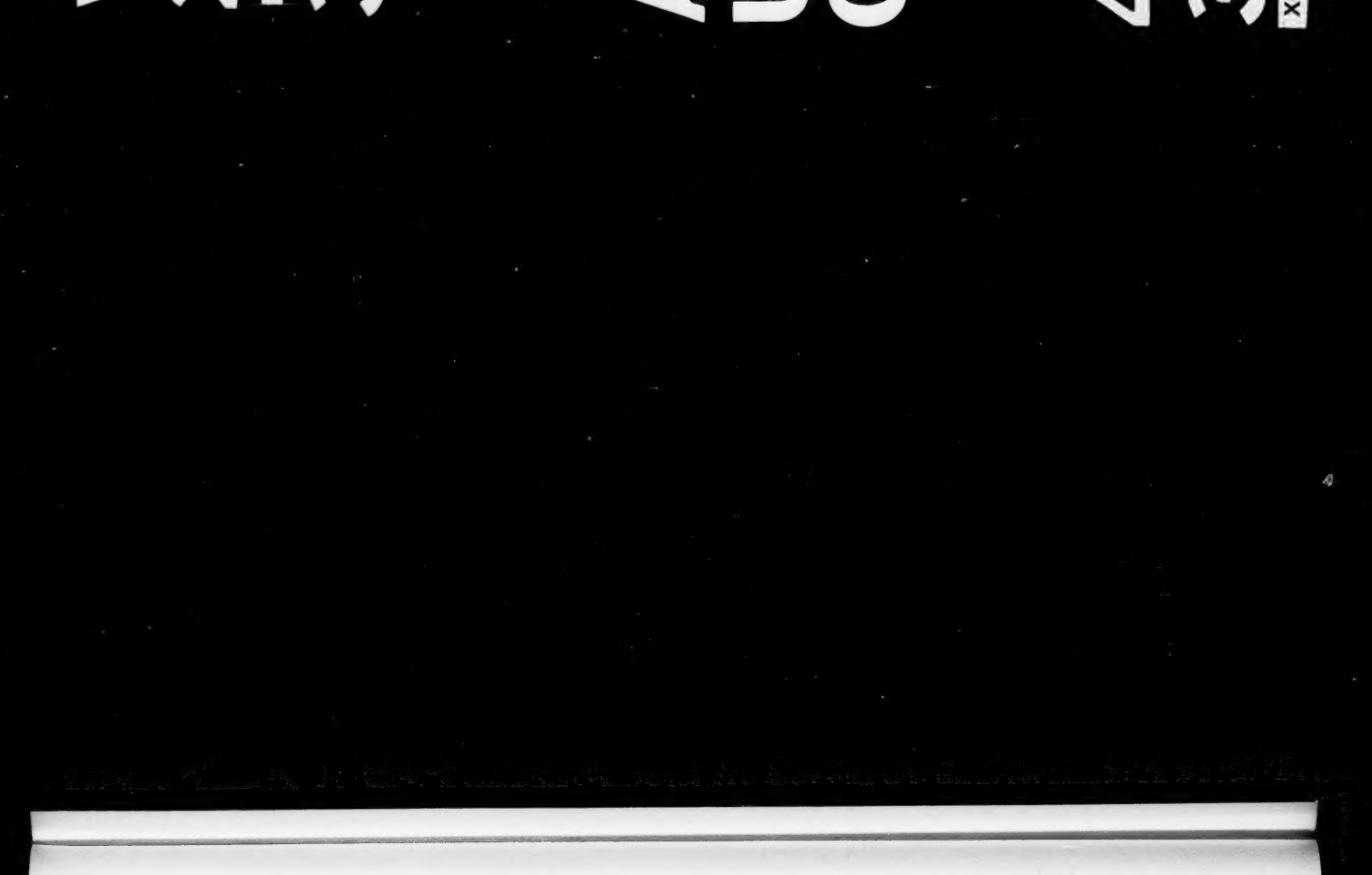
H. H. Stonaker, formerly with the regional swine breeding laboratory has been appointed animal breeding specialist, Colorado Agricultural College.

R. W. Pifer has been made asst. professor, and E. S. McConnell, instructor and asst. in the animal industry department, University of Kentucky.

Alfred Van Wagenen has been appointed associate in animal industry at the Ohio Experiment Station.

Leroy Horn and Irvin F. Edwards have been made asst. professors of animal husbandry at the Utah College of Agriculture, the former on sheep and wool and the latter on meats and beef cattle.

Ivan Watson is now extension animal husbandman, N. Mex. A. & M. College, vice Steve Brock who resigned to join the U. S. Navy. Mr. Watson was formerly on the staff of the Colorado Agricultural College.

R. S. Curtis, formerly head, animal husbandry department, N. C. College of Agriculture and more recently with the Farm Security Administration, is now marketing specialist with the N. C. State Department of Agriculture.

E. F. Rinehart, Idaho extension animal husbandman, A. C. Esplin, Utah sheep extension specialist, and H. H. Smith, Utah beef cattle and swine specialist acted as judges at the 7th Annual Inter-Mountain Fat Stock Show held June 7–8 at Ogden, Utah.